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# Reduction in Chitin Deposition through Acetone Solution of Retinol [(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraen-1-ol)] in Silkworm, *Bombyx mori* (L) [Race-Multivoltine Crossbreed: (PM x CSR2)].

Vitthalrao Bhimasha Khyade, Shubhangi Shankar Pawar alias Shubhangi Madan Sabale

*Science Association, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar Tal. Baramati Dist. Pune – 413115 (India).*

## Abstract

Retinol and Fernasol Methyl Ether (FME) are recognized for the correct functioning of epithelial cells. The ten microliters of various concentrations of acetone solution of Retinol and Fernasol Methyl Ether (FME) 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 Retinol treated larvae was estimated at 120 hours after the fourth moult. Topical application of various concentrations of acetone solutions of FME and Retinol 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 (FME and Retinol)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 of insects through reduction in chitin deposition, it is possible to express the concentration (dose) applied in terms of $ID_{50}$ value. The ID50 value of juvenoid contents of FME and Retinol can be defined as the specific unit (mg/ml), which enable to chitin to deposit fifty percent less in the body wall of larvae (In comparison with untreated control). Accordingly, the $ID_{50}$ value calculated from the "Punyamayee Baramati Dose Response Curves" for FME was found measured 0.08 mg/ml. The $ID_{50}$ value for Retinol was measured 0.095 mg/ml. Acetone soluble juvenoid content of Retinol, the Diterpene 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 and their utilization in sericulture.

**Keywords:** FME; Diterpene; Retinol, ID50 value; Chitin, juvenoids.

## INTRODUCTION

The terpenes are a large and diverse class of organic compounds, produced by a number of plants. The terpenes are also produced by some insects, which emit from their osmeteria. The papilionid larvae are distinguished by presence of osmeteria. The osmeterium is a defensive organ found in all Papilionid larvae, in all stages (4). The osmeterium is situated in the prothoracic segment. It can be everted when the larva feels threatened. Ineverted condition, osmeterium resembles a fleshy forked tongue not unlike a snake tongue and this along with the large eye like spots on the body might be used to startle birds and small reptiles. The osmeterial organ remains inside the body in the thoracic region in an inverted position and is everted when the larva is disturbed in any way emitting a foul, disagreeable odor which serves to repel ants (8); small spiders (7) and mantids (5). The composition of secretion from osmeteria varies from species to species. It contains monoterpene hydrocarbons, sesquiterpenic compounds or a mixture of aliphatic acids and esters. Crossley, A.C. and Waterhouse D.F. (6) studied the fine structure of the osmetrium of *Papilio demoleus libanius* (Fruhstorfer) and found to contain 3 types of specialized cells for synthesis, acid secretion. Lu, Chow-Chin; Yien Shing Chow(19) confirmed the storage of the osmeterial secretion.

The terpenes 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".

One of the animal forms of terpene and vitamin A is retinol. With reference to chemical structure, it is a diterpenoid and an alcohol. Many other forms of Vitamin A are possible through the inter-conversion of retinol, and the retinyl ester derivative of the alcohol serves as the storage form of the vitamin in animals. Retinal form is also known as retinaldehyde and it is essential for vision. Retinoic acid is essential for skin health, teeth re-mineralization and bone growth. All these forms of retinol are are collectively known as retinoids, and possess the structural motif of all-*trans* retinol as a common feature in their structure. The β-ionone ring and a polyunsaturated side chain constitutes chemical structure of retinoids. The side chain of retinoid is composed of four isoprenoid units, with a series of conjugated double bonds which may exist in *trans*- or *cis*-configuration. In animal body, retinol is produced from the hydrolysis of retinyl esters, and from the reduction of retinal. Retinol in turn is ingested in a precursor form; animal sources (liver and eggs) contain retinylesters, whereas plants (carrots, spinach) contain provitamin A carotenoids (these may also be considered simply vitamin A). The Hydrolysis of retinyl esters get results in retinol. The provitamin A carotenoids can be cleaved to produce retinal by carotene dioxygenase in the intestinal mucosa. Retinal, also known as retinaldehyde, can be reversibly reduced to produce retinol or it can be *irreversibly* oxidized to produce retinoic acid, which then cannot function as the vitamin in the eye. Commercial production of retinol typically requires retinal synthesis through reduction of a pentadiene derivative and subsequent acidification/hydrolysis of the resulting isomer to produce retinol. Pure retinol is extremely sensitive to oxidization and is prepared and transported at low temperatures and oxygen free atmospheres. When prepared as a dietary supplement, retinol is stabilized as the ester derivatives retinyl acetate or retinylpalmitate.

Vitamins are the organic compounds required by organism aa vital nutrient in limited amounts. Supplementation of vitamins serve to orchestrate the metabolism. The larvae of silkworm, *Bombyx mori* (L) deserve appreciation for synthesis of silk for it's metamorphosis. Sericultural practices are serving a lot to provide the silk fibre. The silkworm, *Bombyx mori* (L) exert a significant influence on the concept

of insect metamorphosis through it's simple life cycle and efficient utilization of the nutrients from the mulberry, *Morus alba* (L). Interplay of juvenile hormone and moulting hormone in the insect larval body serves to orchestrate the progression of metamorphosis from one instar to next, with moulting hormone regulating the onset and timing of moulting cycle and juvenile hormone regulating the quality of moult (25, 26, 27). During the last larval stadium of holometabolous insects, such as silkworm, *Bombyx mori* (L), a reduction of JH in haemolymph is the necessary step in the initiation of metamorphosis. It has been demonstrated that, haemolymph ecdysteroid and JH level undergo the developmental changes during larval - larval and larval - pupal cycles in silkworm, *Bombyx mori* (L) (3). Juvenoids are well known in prolonging the larval life in the insect and have long been tried for qualitative improvement of silk (10, 20, 24). There is considerable evidence that juvenile hormone mimics occur in plants, which occasionally leads to economically important consequences in the insect development (31, 32). Juvenile hormone active compounds are found in many higher plants, exogenous application through suitable solvents of which exhibited potent activity in the insects (23). Efficient use of available system, the principle of quality improvement made man to use juvenoids for pest control as well as for the silk yield. Use of juvenoids (synthetic, plant derived and animal derived ) in the rearing of silkworm larvae had positive influence, especially in the silk yield (12, 13, 14, 15, 16, 17, 36 ).

Retinoic acid and insect juvenile hormone (JH) are structurally related terpenoids, which are widespread in nature and are involved in much more biological activities including morphogenesis, embryogenesis and cellular differentiation. The retinol is a diterpenoid, a terpenoid derived from a diterpene, which include the compounds with C20 skeleton of the parent diterpene, which has been rearranged or modified by the removal of one or more skeletal atoms (generally, methyl groups) (The retinoids deserve important role in the process of morphogenesis and in immune response in the insects like R. prolixus, suggesting that the molecular mechanism recognize the terpenoid backbone as one of the important structural determinant in insects. Terpenoid hormones seems to act as the morphogens throughout the metazoan. The regulatory activities of terpenoid hormones range from controlling metamorphosis in insects (25) and to determine the germ cell fate in the mammalians (26). In the metamorphosis, the interplay of the juvenile hormone and ecdysone serve to orchestrate the progression from one instar to the next, withecdyosteroid regulating the onset and timing of the moult and JH determining whether the moult would be larval – larval or larval – pupal (12). Phytophagous insects like silkworm, *Bombyx mori* (L) derive their juvenoid nutrients through the plant material available for them (17). Retinol like vitamin nutrients may either be synthesized by the insect tissue or derived from the plant material. Nutrition with vitamins is playing important role in the improvement of growth and development in silkworm, *Bombyx mori* (L).

Juvenoids are known for disruption of normal developmental pattern leading to the deformities in the insects. Interestingly, the silkworm, *Bombyx mori* (L) is known to have a stimulatory influence on the administration of exogenous Juvenoids (JHA) in a appropriate quantities. The specific titer of juvenoids, either topical or through the food, at the specific period of the larval instars of silkworm, *Bombyx mori* (L) are positively reflected into the retention of larval features long enough enabling the larvae to consume maximum quantity of mulberry leaves and to synthesize paramount silk to be used in spinning the qualitative cocoon (15, 36). Diterpene structure, insect juvenoid activity and vitamin nature of Retinol made to plan for the efforts on it's topical application through the acetone to the fifth instar larvae of silkworm, *Bombyx mori* (L) (PM x CSR2).

## MATERIAL AND METHOD

The experimentation was divided into seven steps: Rearing of larval instars of silkworm, *Bombyx mori* (L); Daily bioassay of body wall chitin of fifth instar larvae; Preparation of acetone solutions of Retinol; Grouping the fifth instar larvae and topical application of acetone solution of Retinol; 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 FME and Retinol 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 (15, 18).

**(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 (2, 16). 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 Retinol and FME:**

Retinol and FME 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: 00.000 to 00.160 mg/ml. Likewise, Retinol was dissolved in acetone to get desired concentrations (00.000 to 00.175 mg/ml). FME was used as a "standard Insect Compound responsible for reduction in chitin deposition in insects" for comparison.

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

Soon after the fourth moult, the larvae of fifth instar were grouped into control (Untreated and acetone treated, each one) groups and experimental groups (2 x 35), each with fifty individuals. Ten microliters of each concentration of acetone extractives of FME (as a standard Insect JHA) and Retinol 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; FME Treated and Retinol Treated groups) was carried out at 120 hours after the fourth moult. The method followed for chitin estimation was volumetric (15, 18). 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 experiments 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)(21). 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 centimeter = 00.010 mg/ml concentration of acetone solution. And that for Y- axis, the scale was 1 centimeter= 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 $ID_{50}$ value for given compound. Thus, $ID_{50}$ value for FME and Retinol was calculated through the use of respective dose response curve. The plot of dosages of acetone solution of selected compounds (FME and Retinol) 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".

**RESULTS AND DISCUSSION**

The results pertaining the screening of acetone solution of Fernasol Methyl Ether (FME) and Retinol for JH activity through the changes in pattern of chitin deposition in the integument of fifth instar larvae of silkworm, *Bombyx mori* (L) are presented in Table – 1 to 3 and Fig.1. 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 ).

**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 ) |

1. Each is the mean of three replications.
2. with ± sign in parentheses are the standard deviations.
3. 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 ).

Topical application of ten microlitres of different concentrations of FME and Retinol was found reduction in chitin deposition in the body wall (integument) of the fifth instar larvae of silkworm, *Bombyx mori* (L). 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 compounds in the study (FME and Retinol) and percent reduction in the body wall chitin exhibiting a characteristic Sigmoid form of displacement, which herewith titled as "Punyamayee Baramati Dose Response Curve". The sigmoid curve of pattern of percent reduction in chitin deposition and concentrations of acetone solutions of FME 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, which include: Non-significant; Significant and the most significant. The concentrations of FME namely, 00.000 to 00.060 ppm (mg/ml) of FME were found with non-significant reduction in chitin deposition. The concentrations of FME namely, 00.065 to 00.100 ppm (mg/ml) of FME were found with significant reduction in chitin deposition. And The concentrations of FME namely, 00.105 to 00.160 ppm (mg/ml) of FME were found with the most significant reduction in chitin deposition.

The sigmoid dose response curve for Retinol also exhibited three groups of concentrations of it's acetone solutions. The concentrations of Retinol namely, 00.000 to 00.075 ppm (mg/ml) were found with non-significant reduction in chitin deposition. The concentrations of FME namely, 00.080 to 00.115 ppm (mg/ml) of Retinol were found with significant reduction in chitin deposition. And The concentrations of Retinol namely, 00.120 to 00.175 ppm (mg/ml) of FME were found with the most significant reduction in chitin deposition.

The concentrations (mg/ml) of acetone solutions of FME and Retinol in the study, that inhibit the fifty percent chitin deposition in the body wall of larvae can be calculated by the use of "Punyamayee Baramati Dose Response Curves". This concentration of acetone solution responsible for fifty percent reduction in chitin deposition in the body wall (integument) of fifth instar larvae of silkworm, *Bombyx mori* (L) is herewith termed as $ID_{50}$ value. Accordingly, the $ID_{50}$ values for FME and Retinol were found calculated 00.080and 00.095 units (mg/ml) respectively. Ten microlitres out of thousand microlitres of

each acetone solution is utilized for topical application on individual larva in each group. This study may co-relates the "Finding the efficacy of

**Table – 2: Chitin content of the body wall of the fifth instar larvae of silkworm, *Bombyxmori* (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 * * * (± 3.881) | 00.000 | 100.00 | 20.000 |
| 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 is the mean of three replications;
2. 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, *Bombyxmori* (L) (Race: PM x CSR2) recipient of topical application of various concentration of acetone solution of Retinol 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* (± 4.975) | 18.4 | 00.000 | 00.000 |
| 00.500 | 00.005 | 38.186* (± 5.087) | 18.4 | 00.000 | 00.000 |
| 01.000 | 00.010 | 38.186* (± 4.039) | 18.4 | 00.000 | 00.000 |
| 01.500 | 00.015 | 38.186* (± 4.536) | 18.4 | 00.000 | 00.000 |
| 02.000 | 00.020 | 38.186* (± 4.743) | 18.4 | 00.000 | 00.000 |
| 02.500 | 00.025 | 38.186* (± 4.678) | 18.4 | 00.000 | 00.000 |
| 03.000 | 00.030 | 38.002* (± 6.019) | 18.216 | 01.000 | 00.200 |
| 03.500 | 00.035 | 37.818* (± | 18.032 | 02.000 | 00.400 |

| | | | | | |
|---|---|---|---|---|---|
| | | 4.235) | | | |
| 04.000 | 00.040 | 37.542* (± 6.117) | 17.756 | 03.500 | 00.700 |
| 04.500 | 00.045 | 37.358* (± 5.067) | 17.572 | 04.500 | 00.900 |
| 05.000 | 00.050 | 37.082 (± 6.269) | 17.296 | 06.000 | 01.200 |
| 05.500 | 00.055 | 36.622* (± 6.168) | 16.836 | 08.500 | 01.700 |
| 06.000 | 00.060 | 36.050* (± 5.084) | 16.264 | 11.500 | 02.300 |
| 06.500 | 00.065 | 35.426 (± 5.626) | 15.640 | 15.000 | 03.000 |
| 07.000 | 00.070 | 35.598* (± 6.786) | 14.812 | 19.500 | 03.900 |
| 07.500 | 00.075 | 33.586* (± 6.219) | 13.800 | 25.000 | 05.000 |
| 08.000 | 00.080 | 32.390** (± 6.317) | 12.604 | 31.500 | 06.250 |
| 08.500 | 00.085 | 31.286** (± 5.769) | 11.500 | 37.500 | 07.500 |
| 09.000 | 00.090 | 30.136** (± 5.987) | 10.350 | 43.750 | 08.750 |
| 09.500 | 00.095 | 28.986** (± 4.994) | 09.200 | 50.000 | 10.000 |
| 10.000 | 00.100 | 27.836** (± 5.742) | 08.050 | 56.250 | 11.250 |
| 10.500 | 00.105 | 26.686** (± 5.553) | 06.900 | 62.500 | 12.500 |
| 11.000 | 00.110 | 25.536** (± 6.364) | 05.750 | 68.750 | 13.750 |
| 11.500 | 00.115 | 24.386** (± 4.481) | 04.600 | 75.000 | 15.000 |
| 12.000 | 00.120 | 23.282*** (± 4.493) | 03.496 | 81.000 | 16.200 |
| 12.500 | 00.125 | 22.362*** (± 5.741) | 02.576 | 86.000 | 17.200 |
| 13.000 | 00.130 | 21.810 *** (± 6.213) | 02.024 | 89.000 | 17.800 |
| 13.500 | 00.135 | 21.166 *** (± 5.863) | 01.380 | 92.500 | 18.500 |
| 14.000 | 00.140 | 20.798*** (± 6.087) | 01.012 | 94.500 | 18.900 |
| 14.500 | 00.145 | 20.430*** (± 5.975) | 00.644 | 96.500 | 19.300 |
| 15.000 | 00.150 | 20.154*** (± 4.788) | 00.368 | 98.000 | 19.600 |

| | | | | | |
|---|---|---|---|---|---|
| 15.500 | 00.155 | 20.062*** (± 5.383) | 00.276 | 98.500 | 19.700 |
| 16.000 | 00.160 | 19.970*** (± 4.978) | 00.184 | 99.000 | 19.800 |
| 16.500 | 00.165 | 19.878*** (± 5.975) | 00.092 | 99.500 | 19.900 |
| 17.000 | 00.170 | 19.786*** (± 5.179) | 00.000 | 100.00 | 20.000 |
| 17.500 | 00.175 | 19.786 *** (± 5.882) | 00.000 | 100.00 | 20.000 |

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

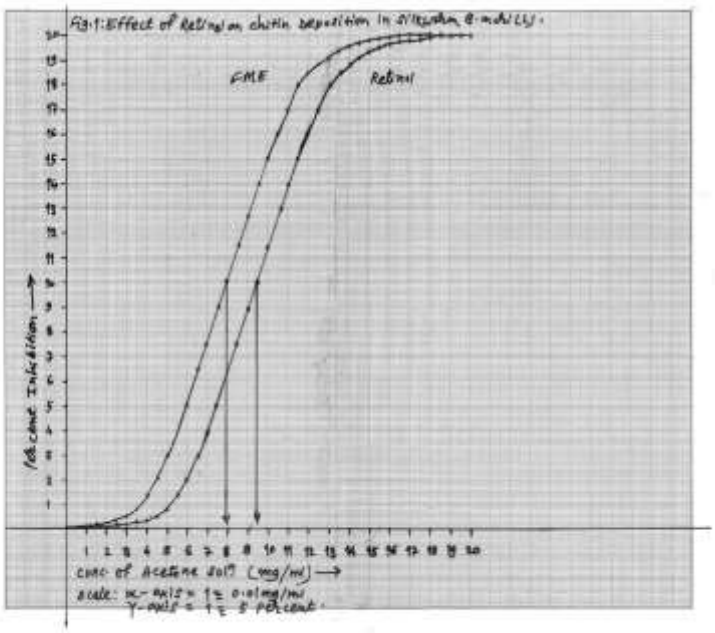


Fig.1: Effect of Retinol on chitin deposition in silkworm, *Bombyxmori* (L) (Race: PM x CSR2).

acetone soluble compound for it's juvenoid activity through reduction in chitin deposition in the of fifth instar larvae of silkworm, *Bombyx mori* (L).

The "Punyamayee Baramati Dose Response Curves" in the study may form baseline platform for estimation of $ID_{50}$ 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 Retinol 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. The diterpene retinol deserve many more cellular and molecular activities

that could potentially underlie it's 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 Retinol to be used for future trials in which the efficacy of retinol 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.

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 (15, 25, 26). 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 (22). 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 retinol; 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 Juvenoid compounds in silkworm larvae (13, 14, 15, 16, 17). Selected doses of selected of diterpenes(like Retinol) 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.The relationship between titer (concentration) of exogenous juvenoid material (acetone solutions of selected FME and Retinol ) & 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 Retinol. 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 retinol 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 Retinol 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 (36). It has been proposed to express the concentration (dosage) of acetone solution or extractives(Juvenoid) topically applied in terms of $ID_{50}$

value. The $ID_{50}$ unit of juvenoid material (in microgram), which deposit fifty percent chitin in the body wall of insect larvae (30). The present attempt is going to help to establish maximum tolerated dose of retinol to be used for future trials in which the efficacy of retinol 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 retinol for the fortified health of larval instars, that could spin the qualitative silky cocoon. The retinol is thus an example of diterpene compounds, which may bridge the areas of sericulture. The Baramati attempt of use of Retinol for topical application to the larval instars of silkworm, *Bombyx mori* (L) hope more efficiently benefitting 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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