



## Utilization of Humulene for the Silkworm Cocoon and Silk yield

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### Abstract

*Humulene sesquiterpene compound with single ring and three units of isoprene. The present attempt was aimed to utilize humulene through acetone for applications (topical) on the second day to the fifth larval stage of double hybrid race of silkworm, Bombyx mori (L). The application humulene through acetone was resulted into yield of qualitative and quantitative silk cocoons and silk fibers. The entire cocoon weight (without floss), weight of shell of silk cocoon, weight of pupae. There was significant improvement in the silk shell percentage (mathematical operation of division of shell weight by the weight of whole cocoon. Resulted quotient multiplied by hundred) and scale of denier of silk through the utilization of spray of humulene solution to the fifth larval stage of double hybrid race of silkworm, Bombyx mori (L). The readings: 2.967\*\* ( $\pm 0.879$ ); 0.843\*\* ( $\pm 0.137$ ); 2.124 and 28.412\*\*\* respectively belong to weight of entire cocoon, weight of silk shell, weight of pupae and the silk shell percentage (ratio of shell to the entire cocoon). The readings: 1489.63\* ( $\pm 229.53$ ); 0.831\*\* ( $\pm 0.118$ ) and 5.020\*\*\* respectively belong to silk fiber length (meter), silk fiber weight (gram) and the scale of denier. As a terpene compound, humulene exhibits probable activity analogous with natural Juvenile Hormone (JH) and may deserve applicable aspects of its utilization as efficient factor for growth of insects like silkworm. The schedule of spraying humulene through acetone should be introduced in the rearing of larval stages of silkworm for yield of qualitative and quantitative silk cocoons and silk fibers.*

**Keywords:** Humulene, Topical spray, Acetone Solvent, *Bombyxmori (L)*

### Introduction

The life on earth is in orchestrate progression with the life of the plants. Credit of the richest sources of nutrients goes to the plants. The animals utilize the food material in the form of plant derived biochemical nutrient-compounds. In a true sense, synthesis of food (in the form of biochemical compounds) by plants through the use of chloroplast is for the purpose of their self-life. The biochemical compound titled, "Humulene" is interplaying to serve in a multitude form in the plant, hops, *Humulus lupulus* (L) for defending the microbial attack. The common plants of hops are belonging to the family: Cannabaceae. Common hops, *Humulus lupulus* (L) are the flowering plant species, native to West-Asia, Europe and the North-America. Hops are perennial, herbaceous, climbers sending shoots in early spring and dies back to a rhizome (cold-hardy) in autumn. Hops are dioecious (male plants and female plants are separate). The cone shaped flowers of female hops, *H. lupulus* (L) are used for preservation and the yield of flavour of the beer. The common hop, *H. lupulus* (L) is used for cultivation for industry of brewing. It occurs naturally in wild regions of a temperate climates<sup>1</sup>. The stem of hop, *Humulus lupulus* (L) is long (6-9m). The surfaces of leaves of *H. lupulus* (L) are not smooth (The leaves are rough surfaced). There is heart shaped petiole (little bit long) at the point of attachment of leaf to the stem. Further the leaf petiole bear lobes (3-5 in number).

Dynamic worldwide trades and the demand of this plant in escalating form are the reasons to increase the significant area of cultivation of *H. lupulus* (L)<sup>2</sup>. The common hop, *H. lupulus* (L) deserve medicinal significance. Therefore, has been reported for the properties of medicine since long times<sup>3,4</sup>. The female cone contains phenolic chemicals beneficial for the treatments of health problems (like: leprosy, obesities, foot-odours and constipations). For the blood purification, contents of this plant are useful. In combination of other drugs, Extractives of *Humulus* species are used as the most significant inducers of sleep. In combination with *Passiflora caerulea* (L), the extractives of *humulus* species are used as sedative agent<sup>5</sup>. There is significant influence on the nervous system through the treatment with *humulus* extractives. This plant also exhibits the medicinal properties including anti-oxidant activity, anti-inflammatory activity, anti-plasmodial activity, anti-viral activity, anti-platelet activity and the properties like: anti-thrombotic, anti-fungal, and anti-bacterial<sup>6-13</sup>. Chemical constituents extracted and identified from hop, *Humulus lupulus* (L) are reported as the most efficient for treatments in the management of symptoms of menopausal fatigue, symptoms of hot flash, symptoms of night sweat, the condition of osteoporosis and for the treatment for different types of conditions of cancer (glioblastoma: cancer type as a growth of brain cells or/ and spinal cord; hepatocellular-carcinoma: cancer type as a growth of liver cells; cancer of mammary glands or

cancer of breast region; cancer of female gonads; cancer of pancreas; cancer of colon region; of thyroid glands; cancer of skin; and cancer of cells of blood)<sup>14-17</sup>. The female hop flowers has glands of lupin specialized in the productions of humulene like compounds, the secondary metabolites. The most significant category of secondary metabolite in the form of secretion of the glands of lupin in the female hop, *H. lupulus* (L) are polyphenols, terpenes, oils (of essential category) and bitter acids<sup>18-21</sup>. The five carbon chains, isoprenes are the functional units of the terpenes, the naturally occurring hydrocarbons of common hops, *Humulus lupulus* (L).

The terpene compounds are occurring naturally as hydrocarbon compounds. They are consisting of chains of five carbons recognized as isoprene units (C<sub>5</sub>H<sub>8</sub>). They are the part and partials of living beings<sup>22</sup>. The terpene compounds are obtained from the cones of common hops, *H. lupulus* (L) and used most significantly in the industries of brewing (most possibly due to the contents terpene compounds). The cones of hops contain 0.5 – 3 percent (of total hop weight). The essential oils contain three types of terpene compounds, which include: Hydrocarbon compounds (60-80%); oxygenated hydrocarbon compounds (20-40%) and organo-sulphur compounds (less than one percent)<sup>11,18</sup>. The group of monoterpene compounds are with ten carbons (C<sub>10</sub>) (Examples: myrcenes; humulenes and sesquiterpene compounds). The chemical nature of Humulene is sesquiterpene-compound. It is with single ring and three units of isoprene. It is also recognized as, alpha-humulene or alpha-caryophyllene. It is sesquiterpene (C<sub>15</sub>H<sub>24</sub>), of category of monocyclic-sesquiterpene-compound. It is having eleven membered ring and consisting of three units of isoprene. It is found firstly in the contents of essential oil of common hops, *H. lupulus* (L). The humulene is reported for anti-inflammatory influences<sup>23,24</sup>. In the year 2015, researchers in country - Brazil identified alpha-Humulene as the most significant and practically active contributors to the property of myrrh (*Commiphora leptophloes* L) leaf oil for repelling the insects, especially against the mosquito, *Ades aegypti* (L)<sup>25</sup>.

Most of the terpene compounds and terpenoid compounds are working for Insect Juvenile Hormones. That is to say, terpene compounds and compounds of terpenoid category achieved the position of Insect-Juvenoids (Juvenile-Hormone-Analogues / JHA). Juvenile-Hormone (JH) is the product of secretion of the neuroendocrine gland: corpora-allata, the part of nervous system in cephalic / head region inside the body-frame of insect species, like silk-worm, *Bombyx mori* (L), use to secrete the Juvenile-Hormone (J.H). Likewise, Moulting-Hormone (MH) is secreted by the neuroendocrine gland: pro-thoracic gland, present in first segment of thorax in the body of insects, like silk-worm, *Bombyx mori* (L)<sup>26</sup>. Specific concentrations (titers) of the J.H and M.H in the body of life stages of silk-worm like insect (silk-worm, *Bombyx.mori* L.) are dealing to proceed to orchestrate the metamorphic progressions. The mechanism of working of these two hormones (Juvenile hormone / JH and hormone of moulting/ M.H) in the body-frame of insect appears

to be exactly opposite to one another. The concentrations or titers of the JH serves to maintain juvenile larval stage. That is to say, the concentrations or titers of the JH use to keep the larval stage into the same stage (age) without further metamorphosis through the events of physiological mechanisms like inhibition of deposition of chitin in the wall of body. Maintaining the larval stage through inhibition of chitin deposition appears to be the most significant action of natural JH and its analogue juvenoids. The Juvenile-Hormone (J.H.) of insect use to retain juvenile stage (younger and younger stage) of insect life. Inhibition of chitin deposition and extension of the larval age is one of the significant effects exerted JH in the larval stage of silkworm. The concentrations or titers of the hormone of moulting (M.H.) serves to proceed to the next phase of life. That is to say, the concentration or titer of the insect Moulting-Hormone (MH) serve to proceed further metamorphosis through many events of physiological actions (including enhancement of deposition of chitin in specific parts of the larval body-frame). In presence of particular / specific concentrations or titers of the hormone of Moulting (M.H.) in the haemolymph (blood) of insect life stage, the mechanism of deposition of chitin in the parts of body-frame appears to be at higher rate. The distinct feature of JH and analogues is inhibition of morphogenetic program at determined in advance by the embryonic constitution (predetermined) and group specific ontogenetic or embryonic developmental positions. It appears that, insect metamorphosis is the outcome of the integrations of fruitful-interplays of specific titers of the JH and MH<sup>27</sup>. The JH and MH, with their specific concentrations are working for the smooth progression of metamorphosis from larval stage to the pupa; from the stage of pupa to the adult stage.

Several herbal compounds; synthetic chemical compounds and biochemicals derived from animal tissue appearing in the list of compounds exhibiting the biochemical features analogous with that of natural Juvenile. Hormone / J.H. of the insects. Williams, in year of 1956, used to designate the plant derived compounds, animal derived compounds and compounds of synthetic nature exhibiting the analogous features and mechanism of working of natural Insect-Juvenile-Hormone (JH) as a special category as Juvenoids<sup>27,28</sup>. Inhibition of chitin deposition and extension of the larval age are the significant effects exerted through the exogenous compounds utilized for topical applications (external spray on the body of silkworm) through appropriate solvent to the larval stages of silkworms of specific age (in hours)<sup>29,30</sup>. Further, the compounds of “juvenoid designation” belong to plant material (herbal compounds) through suitable solvents are reported for the potent natural “Insect-Juvenile-Hormone” (JH) activities through impressive sum of all the metabolic reactions (turnover), alterations of constituencies of metabolites like proteinaceous compounds, lipid compounds, carbohydrate compounds, pool of amino-acids, pool of fatty-acids & chitin (long chain of polymer compound of N-acetyl-glucose-amine)<sup>31-35</sup>. There are several reports on improvement of the status concerned with physiology

of body of larval stages of insects through the reciprocity of exogenous JH and JH analogues (or Juvenoids). Further, the reports are also on utilization of the “Juvenile-Hormone-Analogues” (JHA or juvenoids) for the topical spray (or application) to the individuals of fifth instar silkworms for the qualitative improvements in the silk yield<sup>34-36</sup>.

The chemical class of the “terpenes” is the largest and varied group of compounds of organic nature. The chemical class of the “terpenes” belongs to the diverse groups of the plants and the animals. The chemical class of the “terpenes” belongs to the category of “synthetic” too. The terpene compounds bear strong odor. The terpenes are concerned with protection of the plants through deterring herbivorous animals and through attracting predators and parasites of herbivorous animals<sup>37-40</sup>. Alpha-humulene and caryophyllene are the isomeric compounds. Both (Alpha-humulene and caryophyllene) of them are sesquiterpene compounds and naturally occurring in hopspecies (*H. lupulus*) and *Cannabis sativa* (*Cannabis indica*)<sup>3,4</sup>. There is extensive exploitation of sedative properties of “Humulene” in herbal remedy since a long time. The humulene demonstrated good affinity to Gama Amino Butyric Acid (GABA) and reported for reaching score more than that of paroxetine itself on SERT (serotonin transporter)<sup>41</sup>. Humulene, the sesquiterpene compound is with single ring and three isoprene units. It is also recognized as alpha-humulene or alpha-caryophyllene. It is sesquiterpene (C<sub>15</sub>H<sub>24</sub>) compound and belongs to the “monocyclic category”. It is having eleven membered ring and consisting of three units of isoprene. It firstly investigated as the contents of essential-oil components of common hops, *H. lupulus* (L). The humulene is reported for anti-inflammatory influences<sup>23,24</sup>. In the year 2015, researchers in country - Brazil identified alpha-Humulene as the most significant and compound involved actively as contributor for the repelling the insects through the leaf oil of *Commiphora leptophloes* (L), especially against the mosquito, *Ades aegypti* (L)<sup>25</sup>. In the earlier attempts of authors, topical application (in the form of spray) of humulene was found effecting significant increase in both soluble and total protein contents of silk glands. Therefore, further to analyze the economic parameters of cocoon and silk fiber in silk worm, *Bombyx. mori* (L), present attempt has been sketched out.

## Materials and Methods

The attempt has been completed through the four major steps which include: Silkworm-Rearing; Preparation of acetone solution of humulene; Humulene Treatment; Feeding the larvae with mulberry leaves; Provision of Mountage for Spinning; Cocoon Harvesting; Reeling; Analysis of economic parameters; Data collection and Analysis through the statistical methods.

**Silkworm – Rearing:** The standard sericulture method of silkworm-rearing as prescribed by Krishna swami, *et al.* was followed<sup>42,43</sup>. Silkworm larval stages were fed with fresh leaves obtained from host plant, mulberry, *Morus alba* (L). The mass of eggs of unattached or loose category (disease-free-laying

/D.F.L.) of double hybrid bivoltine race bivoltine [(CSR6 x CSR26) (hybrid bivoltine) x CSR2 x CSR27) (hybrid bivoltine)] was received from silkworm Government Seed Production Center, Gadhinglaj District Kolhapur Maharashtra state (India) through KVK (Address: *Gat / group No: 22/1/B, Road of Solapur to Barshi, Kegaon of North Solapur Tehsil and District- Solapur* (State: Maharashtra, Country: India). The loose mass of eggs (DFL) was processed for the attempt of schedule of rearing (keeping the eggs in black-box for incubation; brushing the hatched larvae from egg mass and transferring them to mulberry-leaf-bed; early in star rearing; late age instars rearing; regular feedings with appropriate mulberry plant (*M. alba* / of variety: M-five); mountage provision for mature fifth instar larvae for spinning the cocoon and cocoon harvesting)<sup>44,45</sup>.

**Preparation of Acetone Solution of humulene:** The humulene was procured from Akshar International, Akshar Chemicals India Private Limited (Hatkesh Udhog Nagar, Mira Road, Mumbai, Maharashtra 401107 India) through local supplier. Acetone solution of humulene of 50 ppm (mg / liter) strength was prepared by dissolving 5 mg powder of humulene in 100 ml acetone solvent. The fresh humulene solution was prepared just few minutes before its utilization.

**Humulene Treatment:** The fifth stage silkworm larvae were utilized for the attempt on humulene treatment. Shortly after lasting moult of fourth number, the larval stages of fifth number were used to transfer in a separate tray (disinfected). The larval stages of fifth number were divided into three groups. Each and every group of the larval stages of fifth number was containing hundred individual larval stages. Each and every group of the larval stages of fifth number of silkworms was in the set of triplicates. Each and every group of the larval stages of fifth number in this attempt consisted of hundred individuals. First group of the fifth stage silkworm larvae in the attempt was considered as: Untreated Control Group. The second group of the fifth stage silkworm larvae in the attempt was considered as: Solvent (Acetone) Treated Control Group. The third group of the fifth stage silkworm larvae in the attempt was considered as: Humulene Treated Group. For the group of hundred fifth stage silkworm larvae, ten milliliters of acetone solution of powder of humulene were used treatment. Acetone solution of humulene of 50ppm (mg / liter) strength was prepared by dissolving 5mg powder of humulene in 100ml acetone solvent. The fresh humulene solution was prepared just few minutes before its utilization. The humulene treatment to the fifth stage silkworm larvae was in the form of uniform spray of ten milliliter solution of humulene. The humulene treatment was executed at forty-eight hours following the process of fourth numbered moulting (on second day of fifth stage silkworm larvae). Hand-sprayer (household category) was used for spraying the solution of humulene in acetone to the fifth stage silkworm larvae. The solvent treated group of larval stages of fifth numbered silkworm received the spray ten milliliters of plain solvent (that is acetone) at forty-eight hours following the fourth moult.

There was no any topical application to the “Untreated control group” of larval stages of fifth numbered silkworms.

**Feeding the larval stages with mulberry leaves:** The fresh and tender leaves of mulberry-tree (*Morus alba* L; Variety: M.5) were used for feeding to the fifth stage silkworm larvae<sup>31</sup>. The schedule of four feedings per day was used to follow. For each and every attempt of feeding, hundred grams of fresh and tender leaves of mulberry tree (*Morus alba* L; Variety: M.5) were used for the group of hundred larval stages of fifth numbered silkworms. The schedule of feeding larval stages of fifth numbered silkworms consisted of: the first feeding- 6.00 AM; the second feeding- 12.00 Noon; the third feeding- 5.00 PM and the fourth feeding- 11.00 PM.

**Mountage Provision for Cocoon-Spinning:** The silk secretion through the mouth opening (spinneret) of mature larval stages of fifth numbered silkworms use to ooze out. At this time, the mature fifth stage silkworm larvae are in search of suitable place for the support. The attempt of provision of suitable and supportive platform (mountage) to the mature larval stages of fifth numbered silkworms is the penultimate (last but one) attempt. In this attempt, mountage is provided to the mature and spinning fifth stage silkworm larvae in the rearing tray (or rearing-bed) directly. In the present attempt, plastic supportive platform (mountage) was supplied. The suitable mountages help for successful spinning the silky cocoon through particular movement of the head by the mature fifth stage silkworm larvae<sup>31</sup>.

**Harvesting the Yield in the form of Silk-Cocoons:** The age of the fifth stage silkworm larvae was counted from the initial time of release of fourth moult to fifty percentage of completion of spinning the silk cocoon. The sixth day was selected for harvesting the yield in the form of silk cocoons. This harvesting was in the form of separation of silk cocoons attached to the plastic supportive platform (mountage) on the day of sixth (counted from the initiation of provision of supportive plastic platform (mountage) for swirling (spinning) the silk cocoon<sup>31</sup>.

**Reeling:** The process concerned with treating the cocoon with boiling water for the purpose of separation of entire (and continuous) silk filament from the silk-cocoon is called as reeling. For purpose of reeling, twenty-five randomly selected silk cocoons were processed for cooking in hot water. The silk fiber from cooked cocoons was separated through the use of country charkha or eprouvette. The process of reeling is exactly opposite to that of process of spinning. In present attempt, during reeling, the silk cocoons were processed for boiling or cooking in water (95-97<sup>0</sup>C). The boiling or cooking the silk cocoons was carried for about ten to twenty minutes. The boiling or cooking the silk cocoons boiling or cooking helps for separation of gum like sericin (gum like substance present around the fibroin contents of silk filament). Boiling or cooking the cocoons is to achieve the ease in the process of reeling (without breaks). In present attempt, from each group, at random twenty-five cocoons were selected and utilized for the

purpose of cooking followed by reeling. Charakha or eprouvette machine was utilized for reeling. The advantage of eprouvette is to get the length (in meter) of silk fibre reeled from individual silk cocoon<sup>31</sup>.

**Analysis Parameters and data collection:** The parameters considered for the present attempt include the age of larval stage of fifth instar; weight of entire cocoon; weight of silk-shell; pupal weight (weight of pupa); length and weight of silk fibers (filament) belong to individual cocoon. The age of the fifth stage silkworm larvae was counted from the initial time of release of fourth moult to the fifty percentage of completion of spinning the silk cocoon. The silk cocoons were used to harvest (separation of silk cocoons attached to mountage in the form of the plastic net) on sixth day after the initiation of provision of mountage (in the form of plastic net) for spinning the silk cocoon. Random selection of silk cocoons (number: fifty) from each and every group in the present attempt was made. Twenty-five cocoons were used for economic or commercial parameters (of cocoon) and remaining twenty-five cocoons were used for reeling. Individual silk cocoon was processed for determination of the weight through the use of electronic balance. Weight (in grams) of each and every individual cocoon (entire silk cocoon) was recorded. Floss from each and every individual cocoon of silk was separated (separation of floss from the silk cocoon is called as deflossing). Individual deflossed cocoon weight of was used to record through the use of electronic balance. Each and every cocoon of silk was processed for vertical cut (through the use of sharp blade). The weight of silky shell of individual cocoon was recorded. The weight of pupa was recorded through the use of electronic balance. The reading of silky-shell of respective silky-cocoon was subtracted from weight of respective individual silky-cocoon (deflossed) for knowing the weight of the pupa from individual silky-cocoon. The readings on the weight of entire silky-cocoon (deflossed); weight of shell of individual silky-cocoon and pupal weight were recorded. The commercial or economic parameter of silk cocoon is the shell-ratio of the silky-cocoon. The silky-cocoon shell ratio is nothing but, the percentage of content of actual silk within the individual silky-cocoon. The readings of whole (entire) deflossed cocoon and silky-shell weight were accounted for the mathematical (algebraic) calculation of shell-ratio or silky-shell-percent (content of actual silk from individual cocoon). In present attempt was calculated through the utilization of readings of whole cocoon (deflossed) weight and the silky shell weight. The silky-shell-weight (gram) reading was mathematically (algebraically) divided by reading of whole / entire deflossed cocoon. This mathematical division yields the quotient, which was processed for the multiplication by hundred to get the shell ratio of individual silky cocoon. In seri cultural practices, the percentage of silk in entire cocoon is recognized as shell-ratio. The silky-shell-ratio determines appropriate price for the silky-cocoon-yield<sup>26-28</sup>.

The process concerned with treating the cocoon with boiling water for the purpose to separate the silk-filament (fibre) from

the silky-cocoons is called as reeling. The process concerned with treating the silky-cocoon with boiling water for the purpose to separate individual silk filament (fibre) from the silky-cocoon is called as reeling. For purpose of reeling, twenty-five randomly selected silky cocoons were processed for cooking in hot water. The silk filament (fiber) from cooked cocoons was separated through the use of country charkha or eprouvette. The process of reeling is exactly opposite to that of process of spinning. In present attempt, during reeling, the silky cocoons were processed for boiling or cooking in water (95-97<sup>0</sup>C). The boiling or cooking the silky cocoons was carried for about ten to twenty minutes. The boiling or cooking the silky cocoons Boiling or cooking helps for separation of gum like sericin (gum like substance present around the fibroin contents of silk filament / fibre). Boiling or cooking the silky-cocoons is to achieve the ease in the process of reeling (without breaks). In present attempt, from each group, at random twenty-five silky-cocoons were selected and utilized for the purpose of cooking followed by reeling. Charakha or eprouvette machine was utilized for reeling. The advantage of eprouvette is to get the length (in meter) of silk fibre reeled from individual silk cocoon<sup>31</sup>.

The weight (grams) of silk-filament (fibre) reeled from individual silky-cocoon was recorded through the use of electronic balance. The reading of weight (grams) of silk-filament (fibre) (B) was divided by the reading of length (meters) of silk-filament (fibre) (A). Finally, the quotient thus resulted was multiplied by nine thousand. The figure thus resulted was titled as the denier scale of silk-filament (fibre) from individual silky-cocoon<sup>29-31</sup>. Thus, through the attempt of analysis of characters of silky-cocoon and characters of silk-filament (fibre), the data collected.

**Data Analysis through the use Statistical Methods:** The collection and scrutinization of data collected are of importance with reference to the statistical analysis. Explanation of features of the data (fact of information in digital form) used to analyze; for explorations of the relations of the data belongs to underlying groups; summarizations of association of the data to elemental groups; validity setting up for the proof of the replica (model) and to heed the analytics of prediction are the consequences of any analysis through the methods of statistics. Identification of the trends parameters in the attempt appears to be the sole aim of data analysis<sup>32</sup>. Each and every event of attempt of the present experimentations were reiterated for thrice. The aim of repetitions of the attempt in present experimentation is to obtain the result of category of consistent qualities. Parameters expected in statistics include: mean, standard deviation and percent change. All these parameters were calculated through the use primary data collected in all the attempts. Finally, the data subjected for the statistical analysis. The percent variations and student “t” – tests were considered for knowing the levels of significance<sup>46-48</sup>.

## Results and Discussion

The outcomes dealing with utilization of humulene through acetone solvent for qualitative and quantitative silk yield from silkworm larvae [Race: Bivoltine Double Hybrid] are epitomized (summarized) through tabular form (Table-1) and presented through plotting the graphs (Figure-1,2,3,4,5,6 and 7). The age (hours) of larval stages of fifth numbered silkworm, *Bombyx mori* (L) [Race: (CSR6xCSR26) (hybrid bivoltine) x CSR2xCSR27) (hybrid bivoltine)] of the untreated control group and solvent (acetone) treated group was found recoded 147.53 ( $\pm 2.961$ ) and 147.54 ( $\pm 2.786$ ) respectively. The age (hours) of fifth stage silkworm larvae belong to the group treated with Humulene (through acetone) was found recoded 182.68 ( $\pm 7.958$ ) units with significant improvement (Table-1 and Figure-1 / Graph-1). Extension of the life of insect larvae is the significant feature of exogenous application (in the form of topical spray) of JHA (juvenoids). The entire (whole) cocoon (deflossed) weight (unit: gm) of whole cocoon (deflossed) of the group of untreated control and solvent (acetone) treated group was found recoded 1.948 ( $\pm 0.392$ ) and 1.948 ( $\pm 0.473$ ) respectively. The entire (whole) cocoon (deflossed) weight (gm) of whole cocoon (deflossed) of the group of humulene (through acetone solvent) treated was found recoded 2.967 ( $\pm 0.879$ ) with significant improvement (Table-1 and Figure-2).

The weight (unit: gm) of silk-shell of deflossed cocoon of the groups of untreated control and plane solvent (acetone) treated groups was found recoded 0.409 ( $\pm 0.0722$ ) and 0.400 ( $\pm 0.078$ ) respectively. The weight (unit: gm) of silk-shell of deflossed cocoon of the group of untreated control and solvent (acetone) treated group was found recoded 0.409 ( $\pm 0.0722$ ) and 0.400 ( $\pm 0.078$ ) respectively. The entire (whole) cocoon (deflossed) weight (unit: gm) of whole cocoon (deflossed) of the group of humulene (through acetone solvent) treated was found recoded 0.843 ( $\pm 0.137$ ) with significant improvement (Table-1 and Figure-3). The shell-ratio of deflossed cocoon of the groups of untreated control and solvent (acetone) treated groups was found recoded 20.995. The shell-ratio of deflossed cocoon of the groups of untreated control and solvent (acetone) treated groups was found recoded 28.412 with significant improvement (Table-1 and Figure-4). The length (unit: meters) of silk-filament (fibre) obtained through reeling the cocoons (with floss) of the untreated control groups and solvent (acetone) treated groups was found recoded 1164.29 ( $\pm 103.24$ ) units and 1164.27 ( $\pm 114.39$ ) units respectively. The length (unit: meters) of silk-filament (fibre) obtained through reeling the cocoons (with floss) of the humulene (through acetone solvent) treated groups was found recoded 1489.63 ( $\pm 229.54$ ) units with significant improvements (Table-1 and Figure-5). The weight (unit: gm) of silk-filament (fibre) obtained through the reeling the cocoon (with floss) of the untreated control groups and solvent (acetone) treated groups was found recoded 0.418 ( $\pm 0.089$ ) and 0.418 ( $\pm 0.093$ ) respectively. The weight (unit: gm) of silk-filament (fibre) obtained through the reeling the cocoon (with floss) of the humulene (through acetone solvent) treated groups was found recoded 0.831 ( $\pm 0.118$ ) with significant improvements (Table-1 and Figure-6). The denier scale of

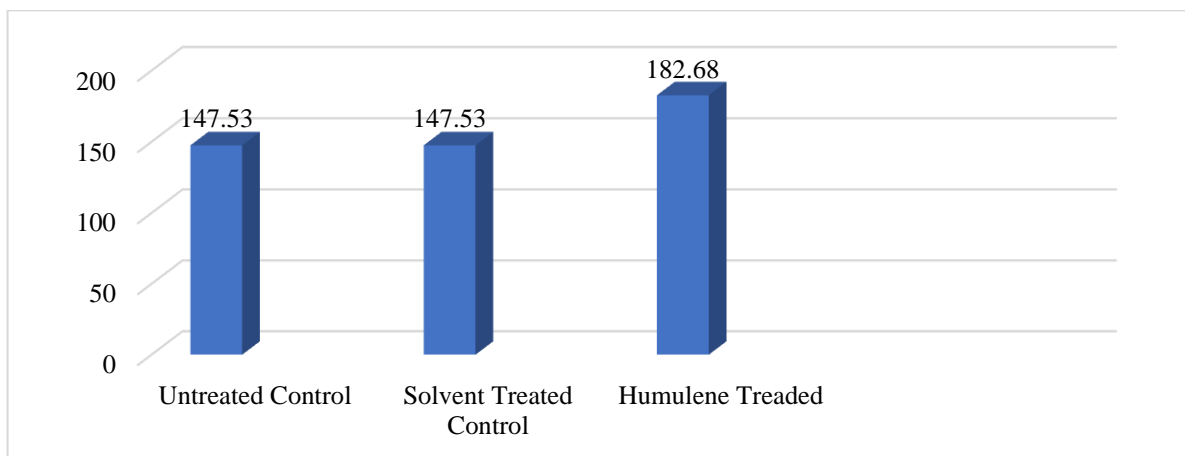
textile filament (fibre) is designed for determination physical quality. The scale of denier for the silk-filament (fibre) obtained from the cocoons (with floss) from the untreated control-group and acetone (solvent) treated group was found recoded 3.231 and 3.231 respectively. The denier scale of textile fibres is designed for determination physical quality. The scale of denier for the silk-filament (fibre) obtained from the cocoons (with floss) from the humulene (through acetone solvent) treated-group was found recoded 5.020 with significant improvement (Table-1 and Figure-7). The foremost and significant feature in sericulture lies in the silk yield in the form of silk-cocoon

prepared (spun) by mature fifth stage silkworm larvae<sup>48-51</sup>. The silk cocoon is the sole source for commercial silk-filament (silk fiber). Most of the compounds of “terpene” category utilized for treatment (for spray /topical application) to the silkworm larvae are mimicking the working mechanism of natural insect the Juvenoids. The significant increase (23.825%) in the age of fifth in star larval stage in present attempt is sufficient to label the Humulene as “Juvenoid compound”. For the fortification of the concept, further studies (on effect of humulene on chitin deposition in insect larval stages) are essential.

**Table-1:** The influence of acetone solution of “Humulene” on the silk-cocoon in silkworm, *Bombyx mori* (L) [Race: Double Hybrid].

Group Parameters	Untreated Control Group	Acetone (Solvent) Treated Control Group	Humulene Treated Group
Age of fifth instar larval stage (Hours)	147.53, (±2.961), 00.000	147.54, (±2.786), 00.000	182.68***, (±7.958), 23.825
Whole Cocoon (deflossed) Weight (gm) (A)	1.948, (±0.392), 00.000	1.948, (±0.473), 00.000	2.967**, (±0.879), 52.310
Shell Weight (gm) (B)	0.409, (±0.072), 00.000	0.409, (±0.078), 00.000	0.843**, (±0.137), 106.11
Shell Ratio [(B÷A) x100]	20.995, 00.000	20.995, 00.000	28.412***, 07.417
Silk Filament Length (meter) (C)	1164.29, 103.24, 00.000	1164.27, (±114.39), 0.000	1489.63*, (±229.54), 27.943
Silk Filament Weight (gm) (D)	0.418, (±0.089), k 00.000	0.418, (±0.093), 00.000	0.831**, (±0.118), 98.800
Denier Scale of Silk Filament [(D ÷ C) x 9000]	3.231, 00.000	3.231, 00.000	5.020***, 01.789

Each figure is the mean of the three replications. Figure with ± sign in the bracket is standard deviation. Figure below the standard deviation is the increase for calculated parameter and percent increase for the others over the control. \*: P < 0.05; \*\*: P < 0.005; \*\*\*: P < 0.01.



**Figure-1:** Influence of Humulene on age of forty-eight hours of fifth stage silkworm larvae.

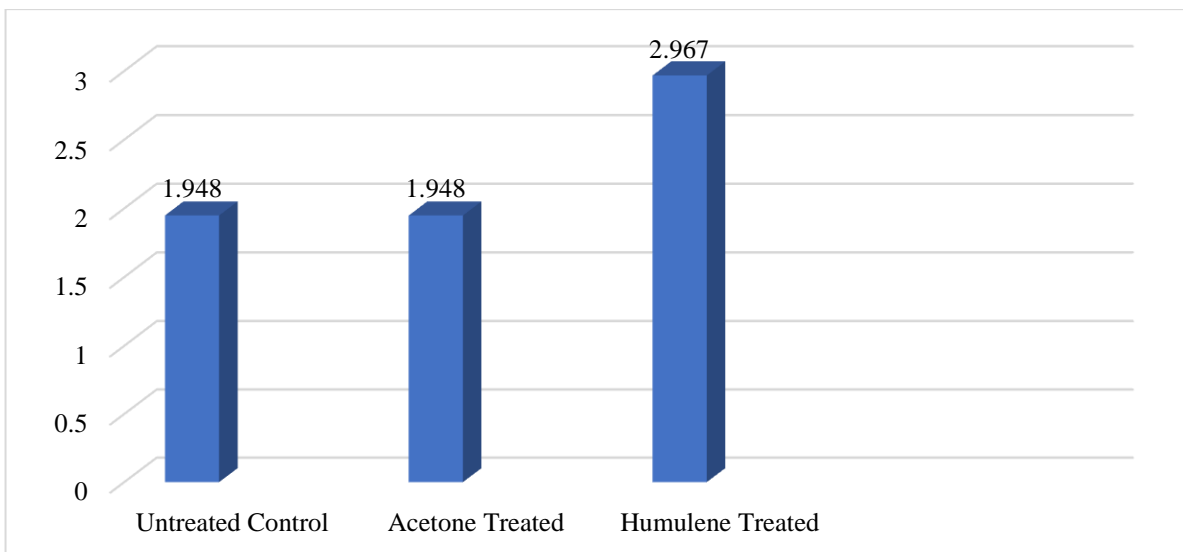


Figure-2: Influence of Acetone Solution on Whole-Cocoon-Weight (gm) in silkworm, *Bombyx mori* (L).

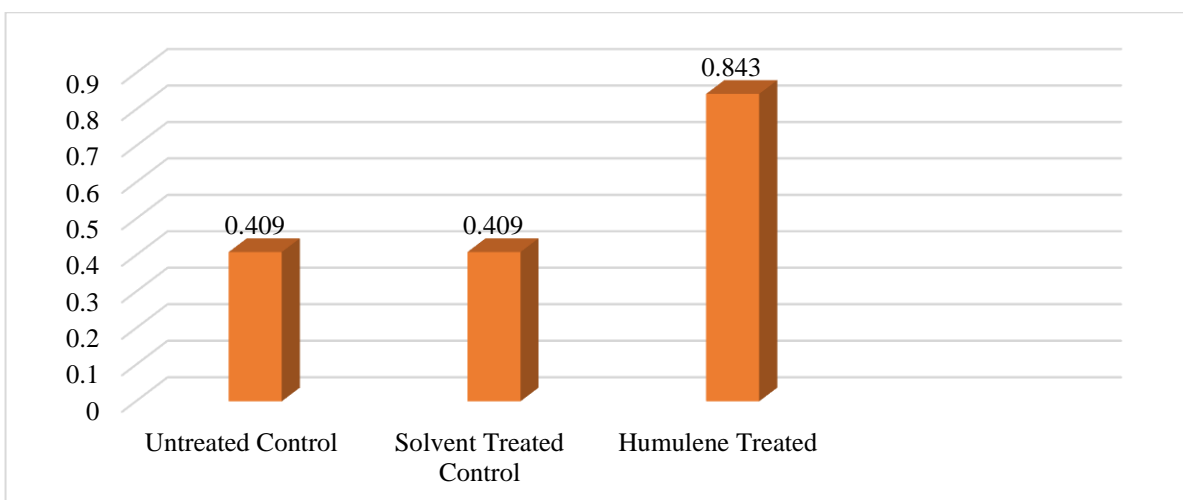


Figure-3: Influence of Humulene on the weight of silk Cocoon-Shell in silkworm, *Bombyx mori* (L).

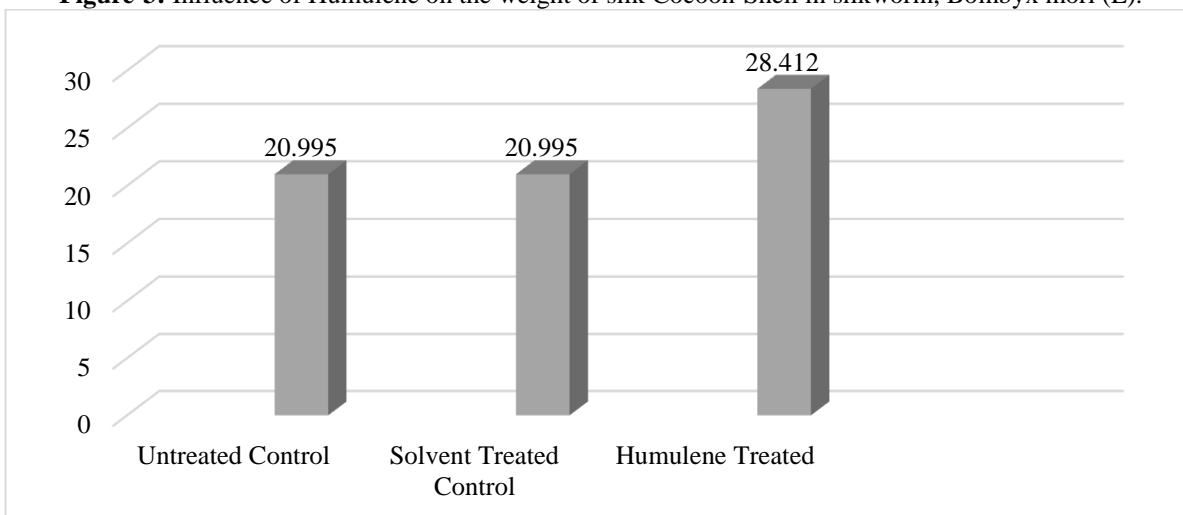
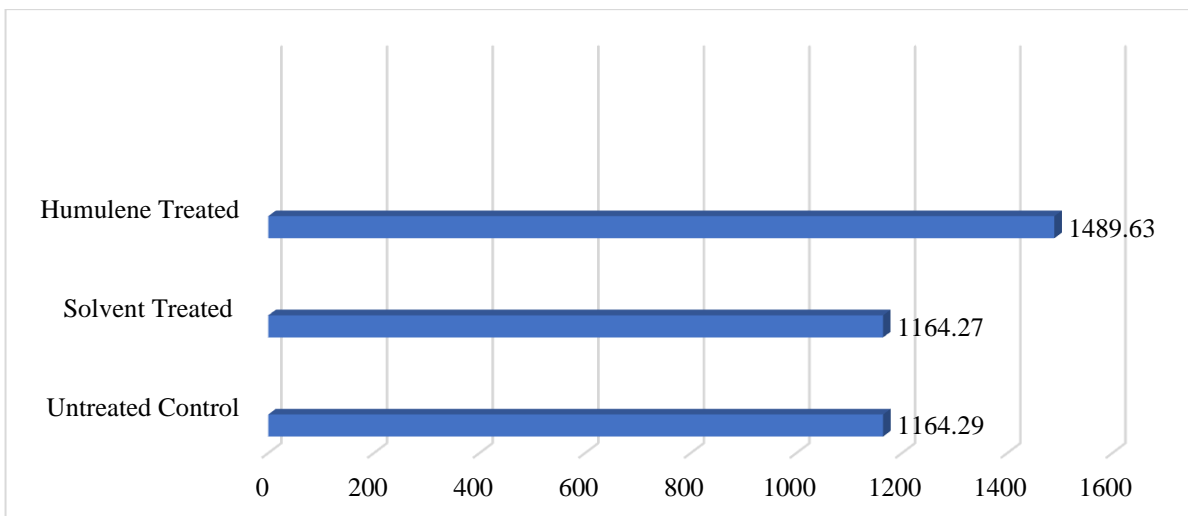
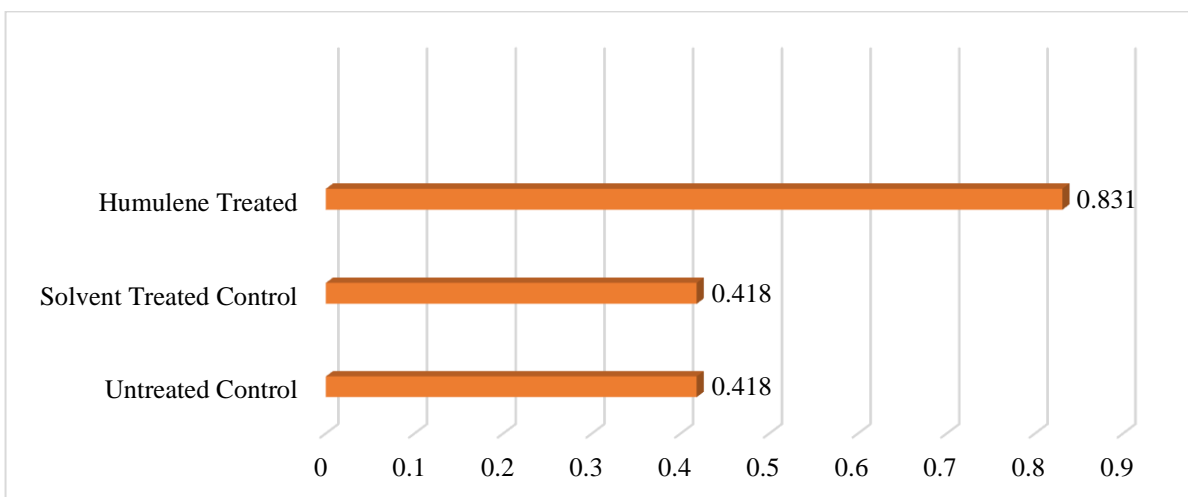


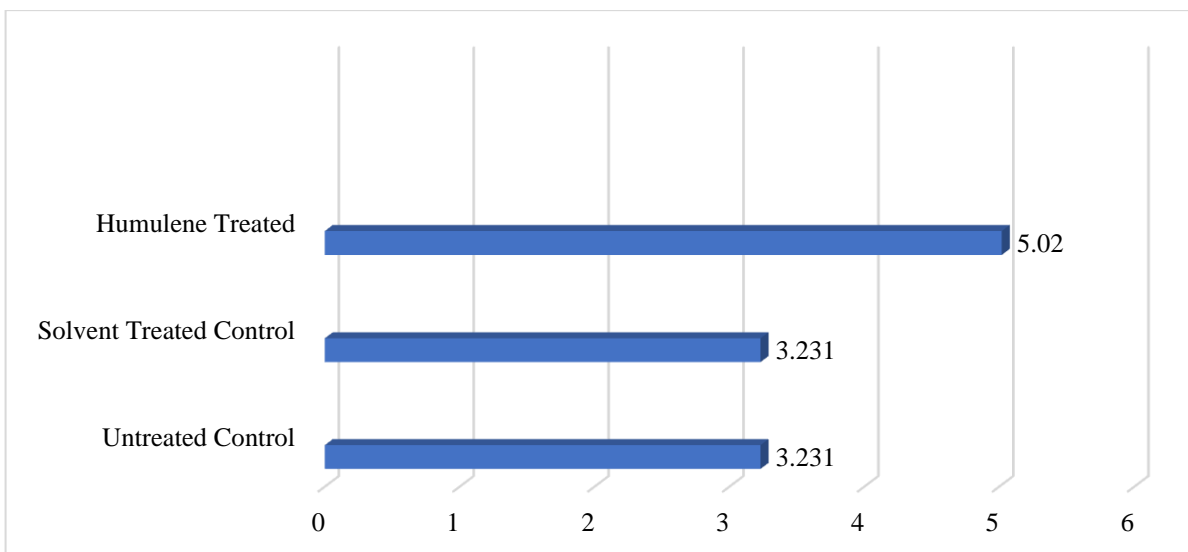
Figure-4: Influence of Humulene on the length (meter) of Silk-Filament obtained through reeling the Cocoons of Silkworm, *Bombyx mori* (L).



**Figure-5:** Influence of Humulene of the Length of Silk Fibers in Silkworm, Bomby mori (L).



**Figure-6:** Influence of Humulene on the Weight (gm) of silk Fiber in Silkworm, Bombyx mori (L).



**Figure-7:** Influence of Humulene on the Denier Scale of Silk Fiber in silkworm, Bombyx mori (L).



There is possibility of influence of humulene (used for topical application to the larval stages of fifth numbered silkworm, *Bombyx mori* (L) [Race: Double Hybrid] at age of forty-eight hours (counted from the release of the fourth moult) on the appetites, consumption and utilization of food-material, secretion of digestive enzymes and absorptions of food digested in the gut. The terpene chemical nature and activities similar to the natural insect juvenile hormone (of humulene), both are responsible for growth of glands of silk in the body of the fifth stage silkworm larvae [Race: Double Hybrid] at accelerated rate. Shell of the cocoon is in fact, made for protection from adverse climatic conditions and for the process of metamorphosis to proceed. The natural and endogenous titer of juvenile hormone (JH) is dealing with stimulation of hypermetabolism<sup>29</sup>. The exogenous titer of juvenoids (from environment or topically applied) is also concerned with stimulation of metabolism at significantly higher rate (hypermetabolism)<sup>31</sup>. The exogenous titers of humulene may have been utilized by the fifth stage silkworm larvae [Race: Double Hybrid] for the synthesis extra silk-protein. Utilization compounds of terpene category with juvenoids activity, chiefly reflect into the significant improvement of qualities of silk cocoon, shell ratio and qualities of silk fiber. The humulene is the most popular sesqui terpene compound. Use of humulene through methanol (as a solvent) for rearing the larval stages of silkworm appears to be much more easy method. Utilization of humulene, monocyclic sesqui terpene compound is going to open a new boulevard (avenue) in seri cultural practices for the quantitative and nuanced research findings (qualitative) of yield of silk.

## Conclusion

The present attempt reports significant influence on the yield of silk through the utilization of acetone solution of humulene for topical applications at forty-eight hours after the fourth moult to the fifth instared larval stages of silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6xCSR26) (hybrid bivoltine)x CSR2xCSR27(hybrid bivoltine)]. The result on the “increase in the age of larval stage of silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6xCSR26) x CSR2xCSR27] in the group received the topical application of acetone solution of humulene” is sufficient to label the humulene compound as, “Insect Juvenoid”.

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