ISSN: 2977-4047

Journal of Life Sciences Research and Reviews

SCIENTIFIC Research and Community

Research Article

Open Access

Qualitative and Quantitative Silk Yield from Silkworm through Utilization of Humulene

Vitthalrao B Khyade

Retired Professor, Department of Zoology, Sharadabai Pawar Mahila Arts, Commerce and Science College, Pune, India.

ABSTRACT

Terpenes and terpenoids are well known to maintain the juvenile condition of silkworm larvae. 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 (ratio of shell to the entire cocoon) 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** (±0.879); 0.843** (±0.137); 2.124 and 28.412*** respectively belong to weight of entire cocoon, weight of silk shell, weigh of pupae and the silk shell percentage (ratio of shell to the entire cocoon). The readings: 1489.63* (±229.53); 0.831** (±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 filaments.

***Corresponding author**

Vitthalrao B Khyade, Retired Professor, Department of Zoology, Sharadabai Pawar Mahila Arts, Commerce and Science College, Pune, India.

Received: October 17, 2024; **Accepted:** October 18, 2024; **Published:** October 30, 2024

Keywords: Humulene, Topical Spray, Acetone Solvent, Bombyx mori (L)

Introduction

Natural bioactive compounds render a number of diversified biological attempts, such as antioxidant, antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory actions, as well as antimutagenicity, anti-carcinogenicity and antiaging effects. The plants as well as animals are essential for human life. 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 [1].

The stem of hop, Humulus lupulus (L) is long (6-9 m). 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) [2].

The common hop, H. lupulus (L) deserve medicinal significance. Therefore, has been reported for the properties of medicine since long times [3,4]. 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 [5].

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 [6-13].

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 of various types of cancer conditions (glioblastoma, hepatocellular-carcinoma, breast-cancer, ovarian-cancer, pancreatic-cancer, colon-cancer, thyroid-cancer, melanoma, and leukemia) [14-17].

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 [18-21]. 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" (C5 H8). They are the part and partials of living beings [22]. 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 percent); oxygenated hydrocarbon compounds (20 – 40 percent) and organo-sulphur compounds (less than one percent) [11, 18].

The group of monoterpene compounds are with ten carbons (C10) (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 (C15H24), 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 [23,24]. 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) [25].

Though bioactivities of some herbals are known through preclinical study attempts, further analytical and clinical research is warranted in order to investigate the bioactive compounds that render effects and their antiaging mode (s) of actions in the silk industries and promotion of silk yield needs to be verified. 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 in the body of insect species, like silk-worm, Bombyx mori (L), use to secrete the Juvenile Hormone (JH). Likewise, "Moulting-Hormone" (MH) is secreted by the neuroendocrine gland: pro-thorasic gland, present in first segment of thorax in the body of insects, like silk-worm, Bombyx mori (L) [26].

Specific concentrations (titers) of the JH and MH in the body of life stages of insects (like silkworm, Bomby 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 hormone / MH) in the body of insects 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 insect "Juvenile Hormone" (JH) retains juvenile 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 (MH) 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). In presence of particular / specific concentrations or titers of the hormone of Moulting (MH) in the haemolymph (blood) of insect life stage, the mechanism of chitin deposition 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 [27].

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 the Juvenile-Hormone / JH of the insects. In the year: 1956, Williams 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" [27,28].

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) [29,30].

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) [31-33].

There are several reports on improvement of the status concerned with physiology of body of larval stages of insects through the recipiency 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 [34-36].

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 [37-40].

Alpha-humulene and caryophyllene are the isomeric compounds. Both (Alpha-humulene and caryophyllene) of them are sesquiterpene compounds and naturally occurring in hop species (H. lupulus) and Cannabis sativa (Cannabis indica) [3,4]. 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) [41].

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 (C15H24) 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 [23,24].

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) [25]. 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 of silkworm, Bombyx mori (L), present attempt has been planned.

Material and Method

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 sericultural method of silkworm-rearing as prescribed by Krishnaswami, et al. was followed [42.43]. Silkworm larval stages were fed with fresh leaves obtained from host plant, mulberry, Morus alba (L). The loose egg mass (disease-free-laying /DFL) of double hybrid bivoltine race bivoltine [(CSR6 x CSR26) x CSR2 x CSR27)] was received from silkworm Government Seed Production Center, Gadhinglaj District Kolhapur Maharashtra state (India) through KVK (Gat No: 22/1/B, Solapur-Barshi Road, At: Khed, Post. Kegaon, Tal: North Solapur, Distt. Solapur Maharashtra state, 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 instar rearing; late age instars rearing; regular feedings with appropriate leaves of mulberry, Morus alba L. M.5 variety; provision of mountage for mature fifth instar larvae for spinning the cocoon and cocoon harvesting) [44,45].

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. Soon after passing fourth molt, the fifth stage silkworm larvae were used to transfer in a separate tray (disinfected). The fifth stage silkworm larvae were divided into three groups. Each group of the fifth stage silkworm larvae was with hundred individuals. Each group of the fifth stage silkworm larvae was in triplicate set. Each group of the fifth stage silkworm larvae in the attempt consisted of hundred individuals. The 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 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. 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 carried out at forty-eight hours after the fourth moult (on second day of fifth stage silkworm larvae). Hand-sprayer (household category) was used for spraying the acetone solution of humulene to the fifth stage silkworm larvae. The solvent treated group of fifth stage silkworm larvae received ten milliliters solvent (acetone) at forty-eight hours after the fourth moult. There was no any topical to the "Untreated control group" of hundred fifth stage silkworm larvae.

Feeding the Larvae with Mulberry Leaves

The fresh and tender leaves of mulberry (Morus alba L; Variety: M.5) were use for feeding to the fifth stage silkworm larvae [31]. The schedule of four feedings per day was used to follow. Hundred grams of fresh and tender leaves of mulberry (Morus alba L; Variety: M.5) were used for the feeding the group of hundred fifth stage silkworm larvae. The schedule of feeding consisted of: the first feeding- 6.00 a.m.; the second feeding- 12.00 noon; the third feeding- 5.00 p.m. and the fourth feeding- 11.00 p.m.

Mountage Provision for Cocoon-Spinning

The silk secretion through the mouth opening (spinneret) of mature fifth stage silkworm 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 mounting the mature fifth stage silkworm

larvae is the 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 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 [31].

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 mountage on sixth day after the initiation of provision of plastic mountage for spinning the silk cocoon [31].

Reeling

The process concerned with treating the cocoon with boiling water for the purpose to separate individual 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 (at 95-970C). 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 [31].

Analysis Parameters and Data Collection

The parameters considered for the present attempt include the age of larval stage of fifth instar; cocoon weight; shell weight; pupal weight; length and weight of silk fibers 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 group in the attempt was made.

Twenty-five cocoons were used for economic or commercial parameters 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. The weight (grams) of each individual entire silk cocoon was recorded. Floss from each individual silk cocoon was separated (separation of floss from the silk cocoon is called as deflossing). Individual deflossed cocoon weight of was recorded through the use of electronic balance. Each silk cocoon was processed for vertical cutting through the use of sharp blade. The weight of silk shell of individual cocoon was recorded pupa was recorded through the use of electronic balance.

The reading of silk-shell of respective cocoon was subtracted from weight of respective individual silk-cocoon (deflossed) for knowing the weight of the pupa from individual silk-cocoon. The readings on the weight of entire silk-cocoon (deflossed); weight of shell of individual silk-cocoon and pupal weight were recorded. The commercial or economic parameter of silk cocoon is the shell-ratio of the silk-cocoon. The silk-cocoon shell ratio is nothing but, the percentage of content of actual silk within the individual silk-cocoon. The readings of whole (entire) deflossed cocoon and silk-shell weight were accounted for the mathematical calculation of shell-ratio or silk-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 silk shell weight.

The silk-shell-weight (gram) reading was mathematically 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 silk cocoon. In sericultural practices, the percentage of silk in entire cocoon is recognized as shell-ratio. The silk-shell-ratio determines appropriate price for the silk-cocoon-yield [26-28].

The process concerned with treating the cocoon with boiling water for the purpose to separate the of silk-fibre from the cocoons is called as reeling. The process concerned with treating the cocoon with boiling water for the purpose to separate individual 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 (at 95-970C).

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 [31].

The weight (grams) of silk-fibre reeled from individual silk-cocoon was recorded through the use of electronic balance. The reading of weight (grams) of silk-fibre (B) was divided by the reading of length (meters) of silk-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 from individual cocoon [29-31]. Thus, through the attempt of analysis of characters of cocoon and characters of silk-filament, 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 relation of the data to underlying groups; validity establishment for the proof of the model and to follow 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 [32]. Each and every events of attempt of the present experimentations were repeated for three times.

The aim of repetitions of the attempts in present experimentation is to get the results 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 [46-48].

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 summarized through table-1 and presented through figures [1-7]. The age (hours) of fifth instar larval stages of silkworm, Bombyx mori (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the untreated control group and solvent (acetone) treated group was found recoded 147.53 (±2.961) and 147.54 (±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 (±7.958) units with significant improvement (table-1 and figure / 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 (gm) of whole cocoon (deflossed) of the group of untreated control and solvent (acetone) treated group was found recoded 1.948 (±0.392) and 1.948 (±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 (±0.879) with significant improvement (table-1 and figure 2).

The weight (gm) of silk-shell of deflossed cocoon of the group of untreated control and solvent (acetone) treated group was found recoded 0.409 (±0.0722) and 0.400 (±0.078) respectively. The weight (gm) of silk-shell of deflossed cocoon of the group of untreated control and solvent (acetone) treated group was found recoded 0.409 (±0.0722) and 0.400 (±0.078) respectively. The entire (whole) cocoon (deflossed) weight (gm) of whole cocoon (deflossed) of the group of humulene (through acetone solvent) treated was found recoded 0.843 (±0.137) with significant improvement (table-1 and figure 3). The shell-ratio of deflossed cocoon of the group of untreated control and solvent (acetone) treated group was found recoded 20.995.

The shell-ratio of deflossed cocoon of the group of untreated control and solvent (acetone) treated group was found recoded 28.412 with significant improvement (table-1 and figure 4). The length (meters) of silk-filament obtained through reeling the cocoons (with floss) of the untreated control group and solvent (acetone) treated group was found recoded 1164.29 (±103.24) units and 1164.27 (±114.39) units respectively. The length (meters) of silk-filament obtained through reeling the cocoons (with floss) of the humulene (through acetone solvent) treated group was found recoded 1489.63 (±229.54) units with significant improvement (table-1 and figure 5).

The weight (gm) of silk-filament obtained through the reeling the cocoon (with floss) of the untreated control group and solvent (acetone) treated group was found recoded 0.418 (±0.089) and 0.418 (±0.093) respectively. The weight (gm) of silk-filament obtained through the reeling the cocoon (with floss) of the humulene (through acetone solvent) treated group was found recoded 0.831 (±0.118) with significant improvement (table-1 and figure 6).

The denier scale of textile fibre is designed for determination physical quality. The scale of denier for the silk-filament 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 fibre is designed for determination physical quality. The scale of denier for the silk-filament 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 [48-53]. The silk cocoon is the sole source for commercial 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 percentage) in the age of fifth instar 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) 00.000	1489.63* (±229.54) 27.943
Silk Filament Weight (gm) (D)	0.418 (±0.089) 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

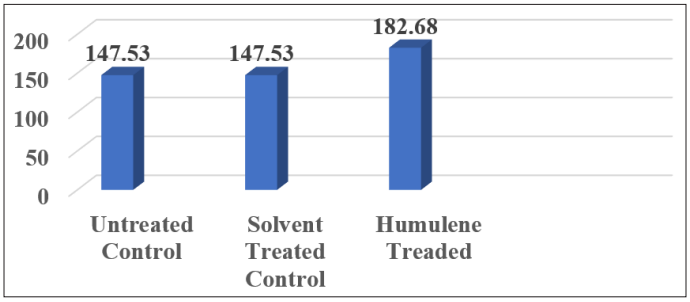

Figure 1: Influence of Humulune on age of forty-eight hours of fifth stage silkworm larvae

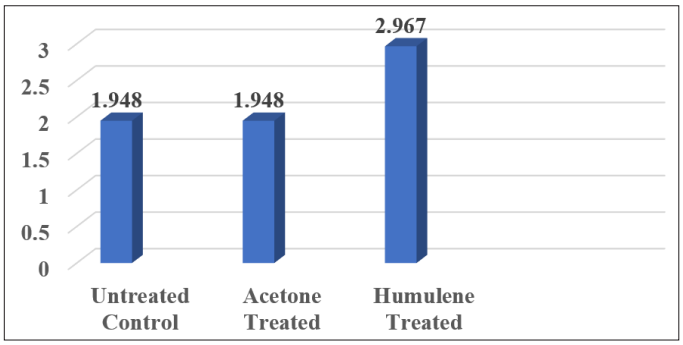

Figure 2: Influence of Acetone Solution on Whole-Cocoon-Weight (gm) in silkworm, Bombyx mori (L)

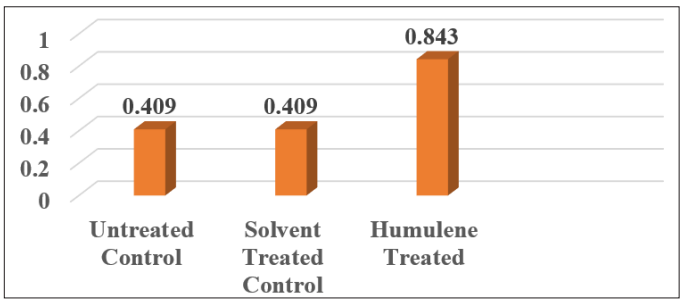

Figure 3: Influence of Humulene on the weight of silk Cocoon-Shell in silkworm, Bombyx mori (L).

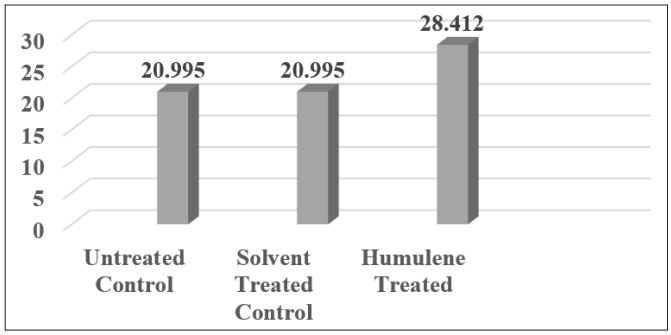

Figure 4: Influence of Humulene on the shell ratio of the Cocoons of Silkworm, Bombyx mori (L)

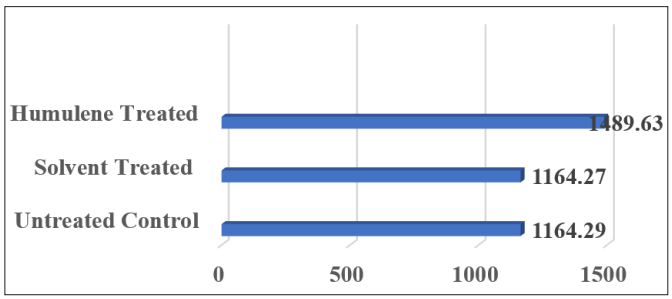

Figure 5: Influence of Humulene of the Length of Silk Fibers in Silkworm, Bomby mori (L)

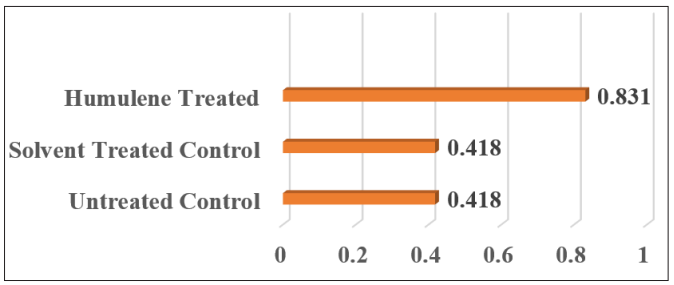

Figure 6: Influence of Humulene on the Weight (gm) of silk Fiber in Silkworm, Bombyx mori (L).

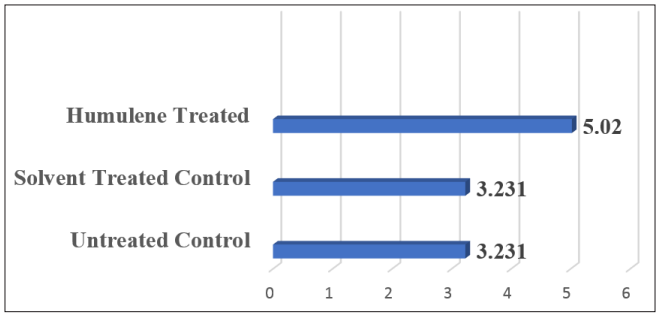

Figure 7: Influence of Humulene on the Denier Scale of Silk Fiber in silkworm, Bombyx mori (L).

There is possibility of influence of humulene (used for topical application to the fifth stage silkworm larvae [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 [29].

The exogenous titer of juvenoids (from environment or topically applied) is also concerned with stimulation of metabolism at significantly higher rate (hypermetabolism) [31]. 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 sesquiterpene 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 sesquiterpene compound is going to open a new avenue in sericultural practices for the quantitative and qualitative 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 - (CSR6 x CSR26) x CSR2 x CSR27]. The result on the "increase in the age of larval stage of silkworm, Bombyx mori (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27] in the group received the topical application of acetone solution of humulene" is sufficient to label the humulene compound as, "Insect Juvenoid".

Acknowledgement

The academic support received from the administrative staff Sharadabai Pawar Mahila Mahavidyalaya, Sharadanagar Baramati and International Science Community Association deserve appreciations and exert a grand salutary influence.

References

1. Biancardi E and Wagner T (1989) ll luppolo da birra in Italia. Annali dell'Istituto Sperimentale per le Colture Industriali 21: 1-59.
2. McCallum JL, Nabuurs MH, Gallant ST, Kirby CW and Mills AAS (2019) Phytochemical characterization of wild hops (Humulus lupulus ssp. lupuloides) germplasm resources from the maritimes region of Canada. Frontiers in Plant Science 10 https://doi.org/10.3389/fpls.2019.01438.
3. Zanoli P and Zavatti M (2008) Pharmacognostic and pharmacological profile of Humulus lupulus L. Journal of Ethnopharmacology 116: 383-396.
4. Tripti Singh and Ashwani Mathur (2024) Humulus lupulus, Plant of Economic and Therapeutic Importance. Current Applied Science and Technology 20: 1-17.
5. Brattström A (2009) Humulus Lupulus (hops), is there any evidence for central nervous effects related to sleep? Acta Horticulturae 848: 173-178.
6. Tagashira M, Watanabe M and Uemitsu N (1995) Antioxidative activity of hop bitter acids and their analogues. Bioscience, Biotechnology, and Biochemistry 59: 740-742.
7. Yamamoto K, Wang J, Yamamoto S and Tobe H (2000) Suppression of cyclooxygenase-2 gene transcription by humulon of beer hop extract studied with reference to glucocorticoid. FEBS Letters 465: 103-106.
8. Liu M, Hansen PE, Wang G, Qiu L, Dong J, et al. (2015) Pharmacological profile of xanthohumol, a prenylated flavonoid from hops (Humulus lupulus). Molecules 20: 754-779.
9. Lin M, Xiang D, Chen X and Huo H (2019) Role of Characteristic Components of Humulus lupulus in Promoting Human Health. Journal of Agricultural and Food Chemistry 67: 8291-8302.
10. Simpson WJ and Smith AR (1992) Factors affecting antibacterial activity of hop compounds and their derivatives. The Journal of Applied Bacteriology 72: 327-334.
11. Hrncic MK, Spaninger E, Kosir IJ, Knez Z and Bren U (2019) Hop compounds: extraction techniques, chemical analyses, antioxidative, antimicrobial, and anticarcinogenic effects. Nutrients 11 https://doi.org/10.3390/nu11020257.
12. Muzykiewicz A, Nowak A, Zielonka-Brzezicka J, Florkowska K, Duchnik W, et al. (2019) Comparison of antioxidant activity of extracts of hop leaves harvested in different years. Herba Polonica 65: 1-9.
13. Carbone K and Gervasi F (2022) An updated review of the genus Humulus: A valuable source of bioactive compounds for health and disease prevention. Plants 11 http://doi.org/10.3390/plants11243434.
14. Van Cleemput MV, Cattoor K, De Bosscher K, Haegeman G, De Keukeleire D, et al. (2009) Hop (Humulus lupulus)-derived bitter acids as multipotent bioactive compounds. Journal of Natural Products 72: 1220-1230.
15. Miranda CL, Stevens JF, Helmrich A, Henderson MC, Rodriguez RJ, et al. (1999) Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (Humulus lupulus) in human cancer cell lines. Food and Chemical Toxicology 37: 271-285.
16. Shimamura M, Hazato T, Ashino H, Yamamoto Y, Iwasaki E, et al. (2001) Inhibition of angiogenesis by humulone, a bitter acid from beer hop. Biochemical and Biophysical Research Communications 289: 220-224.
17. Chen WJ and Lin JK (2004) Mechanisms of cancer chemoprevention by hop bitter acids (beer aroma) through induction of apoptosis mediated by Fas and caspase cascades.

Journal of Agricultural and Food Chemistry 52: 55-64.
18. Krofta K, Hervert J, Mikyška A and Dušek M (2019) Hop beta acids - from cones to beer. Acta Horticulturae 1236: 15-22.
19. Tursun E, Li Z and Aisa AJ (2021) Isolation and identification of soft resins from Humulus lupulus L. Industrial Crops and Products 172 https://doi.org/10.1016/j.indcrop.2021.114014.
20. Forino M, Pace S, Chianese G, Santagostini L, Werner M, et al. (2016) Humudifucol and bioactive prenylated polyphenols from hops (Humulus lupulus cv. "Cascade"). Journal of Natural Products 79: 590-597.
21. Lin M, Xiang D, Chen X and Huo H (2019) Role of characteristic components of humulus lupulus in promoting human health. Journal of Agricultural and Food Chemistry 67: 8291- 8302.
22. Aldred EM, Buck C and Vall K (2009) Terpenes. In: E.M. Aldred, C. Buck and K. Vall, eds. Pharmacology. Edinburgh: Churchill Livingstone 167-174.
23. Passosa GF, Fernandesa ES (2007) Anti-inflammatory and anti-allergic properties of the essential oil and active compounds from Cordia verbenacea. Journal of Ethnopharmacology 110: 323-333.
24. Fernandes ES, Passos GF, Medeiros R, da Cunha FM, Ferreira J, et al. (2007) Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of Cordia verbenacea. European Journal of Pharmacology 569: 228-236.
25. Santos da Silva RC, Milet-Pinheiro P, Bezerra da Silva PC, Gomes da Silva A, Vanusa da Silva M, et al. (2015). Boudko, Dmitri (ed.). €-Caryophyllene and alpha Humulene: Ades aegypti Oviposition Deterrents Elucidated by Gas Chromotography-Electrophysiological Assay of Commiphora leptophloeos Leaf Oil". Plosone 10: e0144586.
26. Zaoral M and Slama K (1970) Peptides with juvenile hormone activity. Science170: 92-93.
27. Williams CM (1956) The Juvenile Hormone of Insects. Nature178: 212-213.
28. Slama K (1971) Insect juvenile hormone analogues. Ann. Rev. Biochem 40: 1079-1102.
29. Gopakumar B, Ambika B and Prabhu VKK (1977). Juvenomimetic activity in some south Indian plants and their probable cause of this activity in Morus alba (L). Entomon 2: 259-261.
30. Khyade VB, Patil SB, Khyade SV and Bhawane GP (2002) Influence of acetone maceratives of Vitis vinifera (L) on the larval parameters of silk worm, Bombyx mori (L). Indian Journal of Comparative Animal Physiology 20: 14-18.
31. Khyade VB (2004). Influence of juvenoids on silk worm, Bombyx mori (L). Ph.D. Thesis, Shivaji University, Kolhapur, India.
32. Williams CM (1956) The Juvenile Hormone of Insects. Nature 178: 212-213.
33. Zaoral M and Slama K (1970) Peptides with juvenile hormone activity. Science 170: 92-93.
34. Slama K (1971) Insect juvenile hormone analogues. Ann. Rev. Biochem 40: 1079-1102.
35. Gopakumar B, Ambika B and Prabhu VKK (1977) Juvenomimetic activity in some south Indian plants and their probable cause of this activity in Morus alba (L). Entomon 2: 259-261.
36. Khyade VB, Patil SB, Khyade SV and Bhawane GP (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.
37. Mamatha DN, Nagalakshmma K and Rajeshwara Rao M (1999). Impact of selected Juvenile Hormone Mimics on the organic constituents of silk worm, Bombyx mori (L).
38. Martin DM, Gershenzon J, Bohlmann J (July 2003) Induction of Volatile Terpene Biosynthesis and Diurnal Emission by Methyl Jasmonate in Foliage of Norway Spruce. Plant Physiology 132: 1586-1599.
39. Pichersky E (10 February 2006) Biosynthesis of Plant Volatiles: Nature's Diversity and Ingenuity. Science 311: 808-811.
40. 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: 68-90.
41. Amany Belal, Mohammed S Elballal, Ahmed A Al-karmalawy, Ahmed HE Hassan, Eun Joo Roh (2024) Exploring the sedative properties of natural molecules from hop cones (Humulus lupulus) as promising natural anxiolytics through GABA receptors and the human serotonin transporter. Frontiers in Chemistry. 10 July, 2024. Sec. Theoretical and Computational Chemistry 12 http://doi.org/10.3389/fchem.2024.1425485.
42. Krishnaswami S, Narasimhana MN, Suryanarayana SK and Kumaraj S (1978) Sericulture Manual –ll: Silk worm Rearing. F A O, United Nation's Rome: 131.
43. Khyade VB, Ghate DP and Sarwade JP (2006) Effect of methoprene on silk worm, Bombyxmori (L). Journal of Zoological Society of India: Environment and Development 49-60.
44. VB Khyade, Sonali S Machale, JP Sarwade, SB Patil and Sadhana H Deshpande (2006) Screening of plant extractives for juvenoid activity in silk worm, Bombyx mori(L). Journal of Zoological Society of India: Environment and Development 61-77.
45. Vitthalrao B Khyade, Poonam M Patil, Kalyani R Jaybhay, Rasika G Gaikwad, Ganga V Mhamane, et al. (2007) Effect of digoxin on economic parameters of silk worm, Bombyx mori (L). Journal of Zoological Society of India: Bioinformatics 23- 31.
46. Norman TJ and Baily (1955) Some Problems in the Statistical Analysis of Epidemic Data. Statistical Methodology (Journal of Royal Statistical Society) First published: January 1955 https://doi.org/10.1111/j.2517-6161.1955.tb00178.x https://rss.onlinelibrary.wiley.com/doi/pdf/10.1111/j.2517-6161.1955.tb00178.x#accessDenialLayout.
47. Vitthalrao B Khyade and Manfred Eigen (2018) Key Role of Statistics for the Fortification of Concepts in Agricultural Studies. International Academic Journal of Innovative Research 5: 32-46.
48. Vitthalrao B Khyade and Sidney Altman (2018) Use of Herbal Terpenoid for topical application to fifth instars of silkworm, Bombyx mori (L). International Academic Journal of Science and Engineering 5 www.iaiest.com http://iaiest.com/journals/international-academic-journal-of-science-and-engineering/volume-5-issue-3-july-september2018/.
49. Vitthalrao B Khyade and Sidney Altman (2018) Use of Herbal Terpenoid for topical application to fifth instars of silkworm, Bombyx mori (L). International Academic Journal of Science and Engineering 5www.iaiest.com http://iaiest.com/journals/international-academic-journal-of-science-and-engineering/volume-5-issue-3-july-september2018/.
50. Manali Ramesh Rao Shinde, Seema Karna Dongare, Vitthalrao Bhimasha Khyade (2018) Qualitative Silk Cocoons in Silkworm, Bombyx Mori (L) Through the Topical Application of Acetone Macerative of Powder of Ganoderma Fruiting Body And Acetone Solution of Its Triterpenoid (Lucidone

–D). International Journal of Scientific Research in Chemistry 3: 17-34.

51. Manisha Mahendra Nalwade, Kajal Appasaheb Pondkule, Vitthalrao B Khyade (2018) The reflection of feeding the mulberry leaves treated with water solution of seed powder of Syzigium cumini (L) into Profiles of Protein (Total) in the fifth instar larvae of silk worm, Bombyx mori (L) Race - bivoltine, crossbreed: [(CSR6 x CSR26)] x [CSR2 x CSR27)]. International Journal of Scientific Research in Chemistry 3: 68-76.
52. Sampada Ravindra Kakade, Pallavi Ramesh Lonkar, Vitthalrao Bhimasha Khyade (2018) Influence of Aqueous Solution of Agaricus bisporus (L) Treated Mulberry Leaves on the Quality of cocoons and silk filament in silkworm, Bombyx mori (L). International Journal of Business Economics and Management Studies 6: 1-16.
53. Sampada Ravindra Kakade, Pallavi Ramesh Lonkar, Vitthalrao Bhimasha Khyade (2018) Influence of Aqueous Solution of Agaricus bisporus (L) Treated Mulberry Leaves on the Quality of cocoons and silk filament in silkworm, Bombyx mori (L). International Journal of Scientific Research in Chemistry 3: 39-51.