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Efficiency of the Zingiberene for the Qualitative Silk

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ABSTRACT

Zingiberene is a constituent of *Zingiber officinale* (L), structurally monocyclic sesquiterpene, and possesses immense pharmacological activity. The present attempt was aimed to utilize a methanol solution of zingiberene for topical application at forty-eight hours after the fourth molt to the fifth instared larvae of silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)]. The topical application of methanol solution of zingiberene was found significant influence on the parameters of the silk cocoon and silk fiber. The weight of the whole cocoon (deflossed), silk shell weight, the weight of pupa and silk shell percentage or ratio in the group recipient of topical application of methanol solution of zingiberene at 48 hours after the fourth molt was recorded at 2.887** (±0.786); 0.839** (±0.158); 2.048 and 29.061*** respectively. The length of silk fiber (in meter); weight of silk fiber (in gram) and denier scale silk fiber obtained by reeling the cocoons harvested from the group of larvae recipient of topical application of methanol solution of zingiberene at 48 hours after the fourth molt were recorded 1478.36* (±229.53.51); 0.829** (±0.119) and 5.045*** respectively. As a terpene compound, Zingiberene shows probable activity analogous with natural Juvenile Hormone (JH) and may have future aspects of its use as growth promoting agent in silkworm, *Bombyx mori* (L).

Keywords: Zingiberene, Topical application, Methanol Solution, *Bombyx mori (L).*

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INTRODUCTION

Life on earth is in orchestrated progression with the life of the plants. The credit for 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, the synthesis of food (in the form of biochemical compounds) by plants through the use of chloroplast is for their own lives. The biochemical compound entitled, "Zingiberene" is serving a lot in the plant, *Zingiber officinale* (L) for defense against microbial attack. The common ginger plant is a member of the family: Zingiberaceae. Regarding the aspects of chemistry, the zingiberene is a member of class: monocyclic sesquiterpene and subclass: triterpene [1, 2]. The monocyclic sesquiterpene: zingiberene is the product that resulted in the pathway of isoprenoid through farnesyl pyrophosphate (FPP). Later, the farnesyl pyrophosphate (FPP) is processed for rearrangement so as to form nerolidyl-diphosphate. The pyrophosphate group from nerolidyl-diphosphate gets removed naturally and the ring of the compound gets closed. During this process, there is the attachment of carbocation on the tertiary carbon to the ring. Through the process of shifting 1, 3-hydride, there is the formation of a little bit more stable allylic-carbocation. In the last step of the synthesis of zingiberene, the cyclic-allylic-proton gets removed. Consequently, it leads to formation of a double bond. The enzyme: "Zingiberene-Synthase" serve a lot in this process of catalysis of the biochemical reaction of synthesis of zingiberene. The enzyme: "Zingiberene-Synthase" also deserves the role in the synthesis of other monoterpenes and other sesquiterpenes [1, 2]. The zingiberene is the sesquiterpene of a volatile category. Therefore, the zingiberene belongs to the chemical family of bisabolene. Maintaining the capabilities of the animal tissues, without the effects of functional impairments is nothing but the process of active and healthiest ageing in animals [3]. Zingiberene deserves the capability of promoting healthy ageing in animals and prolonging span of the life [4]. Literature of the past three decades reported the medicinal properties (like antioxidation, antiinflammation, antimicrobial action and anticancer activity) of zingiberene, the bioactive compound of ginger, *Zingiber officinale* (L) [5]. Reports on medicinal uses of zingiberene include: health protection, disease prevention, and treatment for diseases concerned with aging through modulation of molecular targets involved in the pathogenesis [6].

Specific concentration or the titer of the insect "Juvenile-Hormone" (JH) & the specific concentration or the titer of the insect "Moulting-Hormone" (MH) in the body of life stages of insects are concerned to proceed to orchestrate the progressions of metamorphosis. "Juvenile-Hormone" (JH) is the product of secretion of the neuroendocrine gland: corpora allata present in the cephalic region in the body of insects, like silk a worm, *Bombyx mori* (L). "Moulting-Hormone" (MH) is the product of secretion of the neuroendocrine gland: pro-thoracic gland, present in the first thoracic segment in the body of insects, like silk-worm, *Bombyx mori* (L) [7]. The mechanism of working of the insect "Juvenile-Hormone" (JH) & the insect "Moulting-Hormone" (MH) appears to be exactly opposite to one another. The concentration or titer of the insect "Juvenile-Hormone" (JH) serves to maintain the juvenile condition of the larval stage. That is to say, the concentration or titer of the insect "Juvenile-Hormone" (JH) use to keep the larval stage in the same stage without further metamorphosis. through many physiological mechanisms including inhibition of chitin deposition. The insect "Juvenile Hormone" (JH) retains juvenile stage of insect life. The concentration or titer of the insect "Moulting-Hormone" (MH) serves to proceed to the next phase of life. That is to say, the concentration or titer of the insect "Moulting-Hormone" (MH) use not to keep the larval stage into the same stage, but proceed further metamorphosis through many physiological mechanisms including inhibition of chitin deposition. In presence of a particular titer of the "Insect-Moulting Hormone" (MH) in the body of the insect life stage, the mechanism of chitin deposition appears to be at a higher rate. Morphogenetic program inhibition at predetermined and group-specific ontogenetic (embryonic) positions appears to be the distinct feature of "Insect-Juvenile-Hormone" (JH) [7]. Metamorphosis in insects is the product of the integration of fruitful-interplay of the specific titer of the "Insect-Juvenile-Hormone" (JH) & the specific titer of the insect "Insect-Moulting-Hormone" (MH).

There are several chemical compounds of plant origin, several chemical compounds of animal origin and several chemical compounds of synthetic nature that are exhibiting the biochemical features of the analogy of natural the "Insect-Juvenile-Hormone" (JH). In the year: 1956, Williams [8] 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". Inhibition of chitin deposition and extension of the larval age is one of the significant effects exerted through the exogenous compound used for topical application through appropriate to the larval stage of silkworms at a specific age. Furthermore, 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 massive turnovers, alteration of constituencies of metabolites like proteins, lipids, carbohydrates, amino-acids, fatty-acids & the chitin too [9-13]. There are several reports on the improvement of the physiological status of a larval body of insect life stages through the recipiency of exogenous "Insect-Juvenile-Hormone" (JH) and the "Juvenile-Hormone-Analogues" (JHA or juvenoids). Further, the reports are also on the use of the “Juvenile-Hormone-Analogues” (JHA or juvenoids) for the topical application to the fifth instar larval stages of the silkworm, *Bombyx mori* (L) for the qualitative improvements in the silk yield [14-17].

The chemical class of the “terpenes” is the largest and most varied group of compounds of organic nature. The chemical class of the “terpenes” belongs to 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 a strong odor. The terpenes are concerned with the protection of the plants by deterring herbivorous animals and attracting predators and parasites of herbivorous animals [18-20]. The Zingiberene is a constituent of *Zingiber officinale* (L), structurally monocyclic sesquiterpene and possessess immense pharmacological activity. The terpene chemical nature of zingiberene made to plan the attempt to utilize the acetone solution for topical application to the larval instars of the silkworm, *Bombyx mori* (L).

MATERIALS AND METHODS

The study was completed through the steps such as the rearing of larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)]; Preparation of methanol Solution of zingiberene; Zingiberene Treatment; Mountage Provision for Cocoon-Spinning; Parameters Analysis for data collection and Data Analysis through the statistical methods.

Rearing of larval stages of the silkworm, Bombyx mori (L)

The sericultural standard methods [21] of rearing of larval stages of the silkworm, in the view of Krishnaswami, *et al*. were followed [13, 22-26]. For the purpose to feed the larval stages of the silkworm, fresh leaves of the host plant, mulberry, *Morus alba* (L) were utilized. The egg mass in the form of disease-free-laying (DFL) was procured from silkworm Government Seed Production Center, Gadhinglaj District Kolhapur Maharashtra state (India) through Agricultural Development Trust, Baramati District Pune Maharashtra state (India). The race of silkworm, *Bombyx mori* (L) selected for the present attempt was a double hybrid bivoltine race (CSR6 x CSR26) x CSR2 x CSR27). The egg mass in the form of disease-free-laying (DFL) was processed for the attempt of rearing (black boxing; rearing of early instars; rearing of late age instars; regular feeding with leaves of mulberry, *Morus alba* L. M.5 variety; provision of mountage for mature fifth instar larvae for spinning the cocoon and cocoon harvesting).

Preparation of methanol Solution of zingiberene

The zingiberene was procured from Akshar International, Akshar Chemicals India Private Limited (Hatkesh Udhog Nagar, Mira Road, Mumbai, Maharashtra 401107 India) through a local dealer. 0.5 mg powder of zingiberene was dissolved in 10 ml methanol solvent. The strength of this solution was 50 ppm (50 mg per liter). The fresh zingiberene solution was prepared ten minutes before its utilization.

Zingiberene treatment

The fifth instar larval stages of the silkworm, *Bombyx mori* (L) were utilized for the attempt on zingiberene treatment. At a short time after the fourth moulting-process, the fifth instar larval stages of the silkworm, *Bombyx mori* (L) were used to transfer in a separate tray (disinfected). The fifth instar larval stages of the silkworm, *Bombyx mori* (L) were divided into three groups. Each group of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) was with hundred larvae. Each group of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) was in triplicate set. Each group of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) in the attempt consisted of hundred individuals. The first group of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) in the attempt was considered as: Untreated Control Group. The second group of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) in the attempt was considered as: Solvent Treated Control Group. The third group of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) in the attempt was considered as: Zingiberene Treated Group. For the treatment, for the group of hundred fifth instar larval stages of the silkworm, *Bombyx mori* (L) [bivoltine double hybrid race: (CSR6 x CSR26) x CSR2 x CSR27)], ten milliliters of methanol solution of powder of zingiberene were used. The strength of the methanol solution of powder of zingiberene was 50 ppm (50 mg per liter). The zingiberene treatment to the fifth instar larval stages of the silkworm, *Bombyx mori* (L) was in the form of topical application. Ten-milliliter solution of powder of zingiberene in the methanol was topically applied to the group of hundred-larval stages of the silkworm, *Bombyx mori* (L) at forty-eight hours after the fourth moult. Topical application of the methanol solution of zingiberene to the fifth instar larval stages of the silkworm, *Bombyx mori* (L) was in the form of spray. Household hand sprayer serve the purpose to spray the methanol solution of zingiberene to the fifth instar larval stages of the silkworm, *Bombyx mori* (L). The methanol treated group of hundred fifth instared larval stages of the silkworm, *Bombyx mori* (L) [bivoltine double hybrid race: (CSR6 x CSR26) x CSR2 x CSR27)] received ten milliter solvent methanol at forty-eight hours after the fourth moult. There was no any topical to the "Untreated control group" of hundred fifth instared larval stages of silkworm, *Bombyx mori* (L) [bivoltine double hybrid race: (CSR6 x CSR26) x CSR2 x CSR27)]. Feeding to the fifth instared larval stages of silkworm, *Bombyx mori* (L) [bivoltine double hybrid race: (CSR6 x CSR26) x CSR2 x CSR27)] was carried out through the use of fresh tender leaves of mulberry, *Morus alba* (L) (M.5 variety). The schedule of four feedings per day was used to follow at the rate of hundred grams of mulberry leaves for the group of hundred larvae. The schedule of feeding consisted of: the first feeding at 6.00 a.m.; the second feeding- at noon; the third feeding- at 5.00 p.m. and the fourth feeding- at 11.00 p.m.

Mountage provision for cocoon-spinning

The silk secretion from the body of the mature fifth instared larval stages of the silkworm, *Bombyx mori* (L) use to ooze out through the mouth opening. At this time, the mature fifth instared larval stages of silkworm, *Bombyx mori* (L) are in search of a suitable place for the support. The attempt of transfer of mature larvae to the mountage the mature fifth larval stages of the silkworm, *Bombyx mori* (L) appears to be the last but one attempt. In this attempt, mountage is provided to the larval stages of the silkworm, *Bombyx mori* (L) in the rearing tray (or rearing-bed) directly. The plastic mountages were used. The suitable mountages help for successfully spinning the silky cocoon through particular movement of the head by the mature fifth larval stages of the silkworm, *Bombyx mori* (L) [13, 22, 23].

Parameters analysis for data collection

The parameters considered for the present attempt include the age of the larval stage of the fifth instar; cocoon weight; shell weight; pupal weight; length of silk filament reeled from the individual cocoon and weight of silk filament reeled from the individual cocoon. The age of the fifth instared larval stages of the silkworm, *Bombyx mori* (L) was counted from the initial time of the release of the fourth molt to fifty percent of completion of spinning the silk cocoon. The silk cocoons were used to harvest (separation of silk cocoons attached to the plastic mountage) on the sixth day after the initiation of provision of plastic mountage for cocoon spinning. A random selection of fifty cocoons from each group was made. Twenty-five cocoons were used for economic or commercial parameters and the 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 of each silk cocoon was recorded. Deflossing of each individual silk cocoon was carried out. The weight of such deflossed individual silk cocoon was recorded through the use of electronic balance. Each silk cocoon was processed for vertical cutting through the use of a sharp blade. The weight of the silk shell of individual cocoon was recorded. Likewise, the weight of pupa was recorded through the use of electronic balance. For knowing the weight of the pupa from individual silk cocoon, the reading of the weight of silk shell of cocoon was subtracted from weight of respective individual silk cocoon (deflossed). The readings on the weight of entire silk cocoon (deflossed); weight of shell of the individual silk cocoon and pupal weight were recorded. The commercial or economic parameter of the 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 content of actual silk from individual cocoon (The silk cocoon shell ratio) in present attempt was calculated through the utilization of readings of whole cocoon (deflossed) weight and the silk shell weight. The silk shell weight reading was mathematically divided by reading of whole cocoon (deflossed) weight. This mathematical division yields the quotient, which was processed for the multiplication by a hundred to get the shell ratio of individual silk cocoon. In sericulture, this silk shell percentage is called as shell ratio. The appropriate price for the silk cocoon depends on the reading of "Shell Ratio" [19, 24, 25].

The process concerned with treating the cocoon with boiling water for the purpose to separate the silk fiber from the cocoon is called 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 the process of spinning. In the present attempt, during reeling, the silk cocoons were processed for boiling or cooking in water (95-97^{0}C). The process of boiling or cooking the silk cocoons was carried out for about ten to twenty minutes. This process of boiling or cooking of the silk cocoons helps the process of separation of gum like sericin around the fibroin content in silk filament. This process of boiling the cocoons or cooking the cocoons is to achieve ease in the process of reeling (without breaks). Twenty-five randomly selected silk cocoons from each group in the attempt were utilized to reel. The charakha or eprouvette machine was utilized for reeling. The advantage of an eprouvette is to get the length (in meters) of silk fiber reeled from the individual silk cocoon. The weight (grams) of silk fiber reeled from individual silk cocoon was recorded through the use of electronic balance. The reading of weight (grams) of silk fiber (B) was divided by the reading of length (meters) of silk fiber (A). Finally, the quotient was multiplied by nine thousand. The figure thus resulted was titled as the denier scale of silk filament from individual cocoons [26-28]. Thus, through the attempt to the analysis of characters of cocoon and characters of silk filament, the data was collected.

Data analysis through the use of statistical methods

The collection and scrutinization of data collected are of importance with reference to the statistical analysis. Explanation of features (or the nature) of the data used for analysis; exploration of the relation of the data belonging to an underlying group; summarization 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 of the parameters in the attempt appears to be the sole aim of data analysis [29]. All the attempts in the present experimentation were repeated three times. The aim of repetitions of the attempts in the 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 the primary data collected in all the attempts. Finally, the data were subjected to statistical analysis. The percent variations and student "T" – tests were considered for knowing the levels of significance [29-31].

RESULTS AND DISCUSSION

The results dealing with the attempt on the "zingiberene for the silk yield in silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)]" are summarized in **Table 1** and presented in **(Figures 1-5)**. The age (hours) of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the untreated control group and solvent (methanol) treated group was found recoded 147.42 (±2.958) and 147.42 (±2.993) respectively. The age (hours) of the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the zingiberene (through methanol) treated group was found recoded 179.33 (±2.993) with significant improvement **(Table 1 and Figure 1)**. The weight (gm) of the whole cocoon (deflossed) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the untreated control group and solvent (methanol) treated group was found recoded 1.948 (±0.391) and 1.948 (±0.394) respectively. The weight (gm) of the whole cocoon (deflossed) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the zingiberene (through methanol) treated group was found recoded 2.887 (±0.786) with significant improvement **(Table 1 and Figure 2)**. The weight (gm) of the shell of the cocoon (deflossed) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the untreated control group and solvent (methanol) treated group was found recoded 0.408 (±0.073) and 0.408 (±0.079) respectively. The weight (gm) of shell cocoon (deflossed) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the zingiberene (through methanol) treated group was found recoded 0.839 (±0.158) with significant improvement **(Table 1 and Figure 3)**. The shell ratio of cocoon (deflossed) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of both groups (the untreated control group and solvent, methanol treated group) was found recorded 20.944.

Table 1. The Parameters of silk cocoon fiber and silk fiber Spun by Mature fifth instar larvae of silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)] received topical application of methanol solution of zingiberene at 48 hours after the fourth molt

Group / Parameters	Untreated Control Group	Methanol (Solvent) Treated Control Group	Zingiberene Treated Group
Age of fifth instar larval stage (Hours)	147.42 (±2.958) 00.000	147.42 (±2.993) 00.000	179.33*** (±2.958) 21.645
Whole Cocoon (deflossed) Weight (gm)(A)	1.948 (±0.391) 00.000	1.948 (±0.394) 00.000	2.887** (±0.786) 48.203
Shell Weight (gm)(B)	0.408 (±0.073) 00.000	0.408 (±0.079) 00.000	0.839** (±0.158) 105.63
Shell Ratio [(B÷A) x100]	20.944 00.000	20.944 00.000	29.061*** 08.117
Silk Filament Length (meter) (C)	1164.28 (±103.21) 00.000	1164.28 (±114.33) 00.000	1478.63* (±229.53) 26.999
Silk Filament Weight (gm) (D)	0.417 (±0.089) 00.000	0.417 (±0.093) 00.000	0.829** (±0.118) 98.800
Denier Scale of Silk Filament [(D ÷ C) x 9000]	3.226 00.000	3.226 00.000	5.045*** 01.563

-Each figure is the mean of the three replications.
-Figure with ± sign in the bracket is the 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

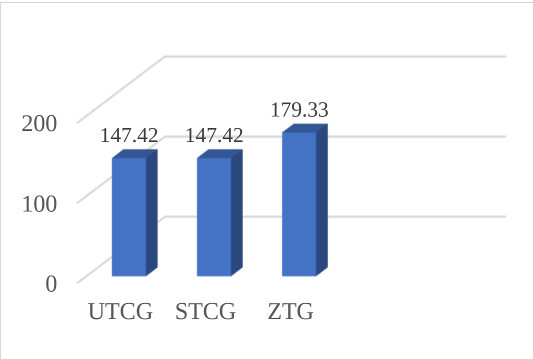

Figure 1. Influence of topical application of methanol solution of zingiberene at 48 hours after the fourth moult on fifth instar larval age (hours) in silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)]
UTCG: Untreated Control Group; STCG: Solvent Treated Control Group; ZTG: Zingiberene Treated Group.

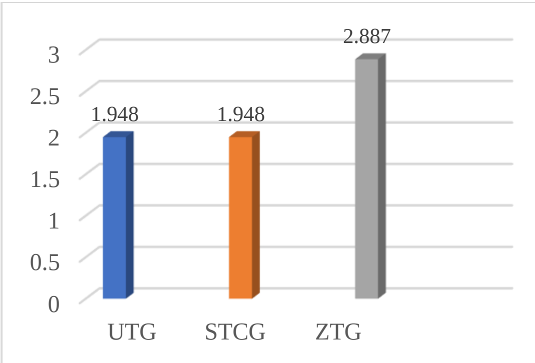

Figure 2. Influence of topical application of methanol solution of zingiberene at 48 hours after the fourth molt on the weight (gm) whole cocoon (deflossed) spun by fifth instar larval stages in silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)]
UTCG: Untreated Control Group; STCG: Solvent Treated Control Group; ZTG: Zingiberene Treated Group.

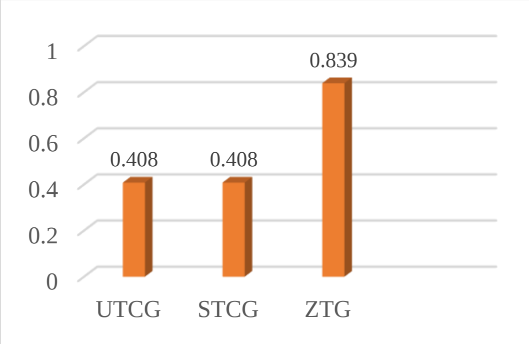

Figure 3. Influence of topical application of methanol solution of zingiberene at 48 hours after the fourth molt on the shell weight (gm) of the cocoon (deflossed) spun by fifth instar larval stages in silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)]

UTCG: Untreated Control Group; STCG: Solvent Treated Control Group; ZTG: Zingiberene Treated Group.

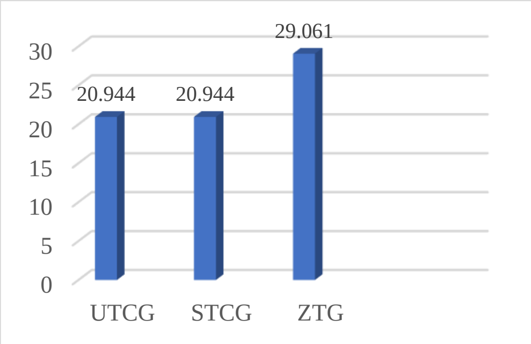

Figure 4. Influence of topical application of methanol solution of zingiberene at 48 hours after the fourth moult on the shell ratio of the cocoon (deflossed) spun by fifth instar larval stage in silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)]

UTCG: Untreated Control Group; STCG: Solvent Treated Control Group; ZTG: Zingiberene Treated Group.

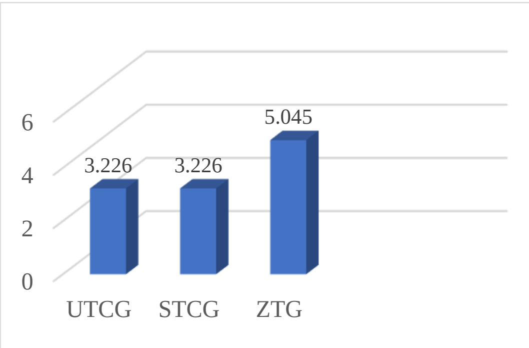

Figure 5. Influence of topical application of methanol solution of zingiberene at 48 hours after the fourth molt on the Denier Scale of silk fiber reeled from cocoon spun by fifth instar larval stage in silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)]

UTCG: Untreated Control Group; STCG: Solvent Treated Control Group; ZTG: Zingiberene Treated Group.

The shell ratio of the cocoon (deflossed) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the zingiberene (through methanol) treated group was found recoded 29.061 with significant improvement **(Table 1 and Figure 4)**.

The length (meters) of silk filament reeled from the cocoon (with floss) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the untreated control group and solvent (methanol) treated group was found recoded 1164.28 (±103.23) and 1164.28 (±114.33) respectively. The length (meters) of silk filament reeled from the cocoon (with floss) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the zingiberene (through methanol) treated group was found recoded 1478.63 (±229.53) with significant improvement **(Table 1)**.

The weight (gm) of silk filament reeled from the cocoon (with floss) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the untreated control group and solvent (methanol) treated group was found recoded 0.417 (±0.089) and 0.417 (±0.093) respectively. The weight (gm) of silk filament reeled from the cocoon (with floss) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the zingiberene (through methanol) treated group was found recoded 0.829 (±0.118) with significant improvement **(Table 1)**.

The denier scale of textile fiber is designed for the determination of physical quality. The denier scale of of silk filament reeled from the cocoon (with floss) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the untreated control group and solvent (methanol) treated group was found recoded 3.226 and 3.226 respectively. The denier scale of silk filament reeled from the cocoon (with floss) spun by the fifth instar larval stages of the silkworm, *Bombyx mori* (L) [Race: (CSR6 x CSR26) x CSR2 x CSR27)] of the zingiberene (through methanol) treated group was found recoded 5.045 with significant improvement **(Table 1 and Figure 5)**.

The silk cocoon spun by the mature fifth instared larval stage of the silkworm, *Bombyx mori* (L) is foremost and significant feature in sericulture

[32-35]. The silk cocoon is the sole source for commercial silk fiber. Most of the compounds of "terpene" category utilized for treating (for topical application to) the larval stages of silkworm are mimicking the working mechanism of natural insect the Juvenoids. The significant increase (21.645 percentage) in the age of fifth instar larval stage in present attempt is sufficient to label the zingiberene as "Juvenoid compound". For the fortification of the concept, further studies (on effect of zingiberene on chitin deposition in insect larval stages) are essential.

There is possibility of influence of zingiberene (used for topical application to the fifth instared larval stages of silkworm, *Bombyx mori* L. [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)] at 48 hours after the fourth molt in the present attempt) on the appetite, secretion of digestive enzymes and absorption of digested food through the gut. The terpene nature and activity of natural insect juvenile hormone, both are responsible for the growth of glands of silk in the body of the fifth instared larval stages of the silkworm, *Bombyx mori* L. [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)] at accelerated rate. In fact, the shell of the cocoon is made for protection from adverse climatic conditions and for metamorphosis to proceed. The natural and endogenous titer of juvenile hormone (JH) (from the endocrine system within the body of silkworm larvae) is concerned for stimulation of hypermetabolism [9, 26, 36, 37]. The exogenous titer of juvenoids (from the environment or topically applied) is also concerned with the stimulation of hypermetabolism [9, 13]. The exogenous titer of zingiberene may be utilized by the fifth instared larval stages of the silkworm, *Bombyx mori* L. [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27)] for the synthesis extra silk protein. Utilization compounds of the terpene category with juvenoids activity, chiefly reflect the significant improvement of qualities of silk cocoon, shell ratio and qualities of silk fiber [38-40]. Hence, zingiberene is the most popular monocyclic sesquiterpene compound. The use of zingiberene through methanol (as a solvent) for rearing the larval stages of silkworm appears to be a much easier method. Utilization of Zingiberene, a monocyclic sesquiterpene compound may open a new avenue in sericultural practices for the quantitative and qualitative yield.

CONCLUSION

The present attempt reports a significant influence on the yield of silk through the utilization of methanol solution of zingiberene for topical applications at forty-eight hours after the fourth mout to the fifth instared larval stages of the silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27]. The result of the "increase in the age of larval stage of the silkworm, *Bombyx mori* (L) [Race: Double Hybrid - (CSR6 x CSR26) x CSR2 x CSR27] in the group received the topical application of methanol solution of zingiberene" is sufficient to label the zingiberene compound as, "Juvenoid".

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