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# Topical Application of Grape Seed Oil and Linalool, and Treating the Mulberry Leaves with Aqueous Solution of Drakshsava Before Feeding the Fifth Instars of Silkworm, *Bombyx mori* (L.) for Qualitative Silk Cocoons

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***Abstract***

*Nutrition quality and health of larval instars exert influence on silk-yield-quality. Ten microliters of Acetone-solutions of Grape-Seed-Oil (10 ml Grape-Seed-Oil dissolved in 90 ml acetone) and acetone solutions of Linalool (100 ppm) were used separately in the present attempt for the topical application to the fifth instar larval stages of silkworm, Bombyx mori (L). Group of larvae fed mulberry leaves treated with aqueous solution Drakshasav; group of larvae topically applied with acetone solutions of Grape-Seed-Oil (10 ml Grape-Seed-Oil dissolved in 90 ml acetone), followed by feeding mulberry leaves treated with aqueous solution Drakshasav were also maintained. Fifth-Instar-Larval-Age (hours) and Tissue-Somatic-Index of silk glands of Untreated-control-group, Grape-Seed-Oil-treatment, linalool-treatment; Drakshasav-treatment and Grape-Seed-Oil-treatment acetone followed Drakshasav-treatment were recorded 145.33 (± 13.786), 31.426; 168.73 (± 13.221), 52.625; 177.46 (± 13.786), 52.728; 162.87 (±14.572), 52.759 and 168.58 (±18.789), 53.854 units, respectively. Shell Ratio of cocoon spun by fifth instar silkworms of untreated-control-group, Grape-Seed-Oil-treatment; Linalool-treatment; Drakshasav-treatment and Grape-Seed-Oil-treatment acetone followed Drakshasav-treatment were recorded 19.422; 23.970; 27.989; 28.048 and 28.378 units, respectively. Denier scale of silk filament spun by fifth instar silkworms of control group, Grape-Seed-Oil treated group; linalool treated group; group fed with mulberry leaves treated with aqueous solution of Drakshasav and group treated with Grape-Seed-Oil treated acetone (topical) followed feeding by mulberry leaves treated with aqueous solution of Drakshasav were recorded 3.243; 4.706; 4.793; 4.882, and 4.948 units, respectively. The range of improvements of tissue somatic index of silk glands (TSI); Shell Ratio of cocoon and Denier scale of silk filament through treatment was 52.625–53.854, 23.970–28.378, and 4.706–4.948. Efficient use of source of juvenoids, like linalool, grape seed oil, and Drakshasav, in desired solvent for treating the silkworm serve to orchestrate the fortification of health through the prevention of the infection of microbial pathogens; extension of larval-age, consumption-and-utilization of nutrition for significant yield of silk product.*

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**Keywords:** Grape-seed-oil, linalool, Drakshasav, tissue somatic index (TSI), shell ratio, Denier scale of silk

## INTRODUCTION

The significant feature of autotrophic and heterotrophic lives on earth is orchestrated progression. Autotrophic plants are serving as innovative and the richest sources of nutrients for the animal lives. Heterotrophic lives (like animals) utilize the nutrients (in the form of functional food material

 

and biochemical nutrient-compounds) and derive energy to lead a successful life [1]. The common grape vine *Vitis vinifera* (L.), the common grape vine, is a species of plant with flowers. The variety of grapes, the process of vinification, grape-maturation, and grape-aging are the factors associated with qualities of aroma of grape-wine. Monoterpenols, particularly linalool, geraniol, and nerol, are responsible for the characteristic floral aroma in grapes [2–5]. In grapes, terpenoids exist both free and as glycosides, being some of the bound terpenoids being released either chemically or by natural β-glycosidase activity of either the grape or of yeasts and bacteria during the vinification phases [6–13]. Linalool is a colorless oil, belongs to "Acyclic Monoterpenoid". In plants, linalool is a volatile metabolite with antimicrobial property [3, 4]. It is used for spraying raisins to help them retain their flavor [14]. The "Drakshasava", ayurvedik tonic is derived from grapes. It is in the form of partial fermentation. Utilization of raisin concentrate is also followed for the preparation of "Drakshasava", ayurvedik tonic. The "Drakshasava" is claimed to be beneficial for ailments (such as conditions of lethargy, weakness-conditions and heat-burnout (or heart-exhaustion). The most significant categories of metabolites and herbal formulations derived from *Vitis vinifera* (L) linalool, grape seed oil (GSO), and "Drakshasava", ayurvedik tonic [15–20]. Many more compounds of herbal origin; compounds of synthetic categories and animal derived biochemicals appearing in the classified list of compounds with features analogous with Insect Juvenile Hormone / J.H. Therefore, further to analyze the influence of acetone solution of grapeseed oil (topical application); acetone solution of linalool (topical application) and aqueous solution of Drakshasav (through mulberry leaves) on economic parameters in silkworm, *Bombyx mori* (L.) (fifth instar larval life duration; weight of cocoon; weight of shell of cocoon; silk shell ratio and denier scale of silk), present attempt has been sketched out [21–28].

## MATERIAL AND METHOD

The experimental procedure was carried out in a structured sequence encompassing several essential steps: rearing of silkworm larval instars, preparation of experimental solutions (acetone-based GSO and linalool solutions, and an aqueous Drakshasav solution), feeding treatments using mulberry leaves, provision of mountage for cocoon spinning, harvesting of cocoons, reeling, followed by the collection and statistical analysis of economic parameters. Rearing of *Bombyx mori* (L.) followed the standard methodology outlined by Krishnaswami et al. [20, 29]. The race employed in this study was a double hybrid: [(CSR6 × CSR26) (bivoltine hybrid)] × [(CSR2 × CSR27) (bivoltine hybrid)]. The test substances—GSO, linalool, and Drakshasav—were procured from Ases Chemical Works (Brahm Bagh, Jalori Gate, Jodhpur-342001, India) via a local distributor. For the treatments, a 10% acetone solution of GSO was prepared by dissolving 10 mg of the oil in 90 ml of acetone. A 100-ppm solution of linalool was obtained by dissolving 10 mg of linalool in 100 ml of acetone. The aqueous solution of Drakshasav was made by mixing 10 ml of Drakshasav in 90 ml of distilled water. All solutions were freshly prepared shortly before use.

Fifth instar larvae, immediately after completing their fourth moult, were separated into disinfected trays for treatment. These larvae were randomly assigned to seven experimental groups, each containing 100 individuals, with three replications per group:

- Untreated control group .
- Acetone-solvent control group.
- Water-solvent control group .
- Topical application of GSO in acetone.
- Topical application of linalool in acetone.
- Feeding with mulberry leaves treated with aqueous Drakshasav.
- Combined treatment: Topical GSO in acetone + feeding with Drakshasav-treated leaves.

The Untreated Control Group received no application. The Acetone Control Group was exposed to a uniform spray of 10 ml plain acetone at 48 hours after the fourth moult. For the GSO group, 10 ml of its acetone solution was uniformly sprayed on larvae using a standard household sprayer at the same 48-hour post-moult interval. Likewise, 10 ml of the linalool solution was applied topically to its respective group using the same method and timing. The sixth group was fed mulberry leaves soaked in the aqueous Drakshasav solution. The seventh group underwent both topical GSO application and dietary Drakshasav exposure through treated mulberry leaves.

 

Each treatment was designed to explore the influence of topical and dietary supplementation on the fifth instar silkworms, with special focus on economic traits related to silk production, which were subsequently subjected to statistical evaluation.

The Drakshasav (through distilled water) treatment to the fifth stage silkworm larvae (hundred) was in the form of feeding mulberry leaves treated with an aqueous solution of Drakshasav. 100 mg of fresh mulberry leaves were kept immersed in 100 ml of aqueous solution of Drakshasav for about an hour. The treated mulberry leaves were decanted and used for feeding to the fifth instar larvae of silkworm at 48 hours after the fourth moult. The solvent treated group was fed with water-treated mulberry leaves.

The seventh experimental group of fifth instar *Bombyx mori* larvae received a combined treatment—first a topical spray of the acetone-based GSO solution, followed by feeding on mulberry leaves that had been treated with an aqueous solution of Drakshasav. Additionally, the acetone solution of linalool was applied via a uniform 10 ml spray to the respective group 48 hours after the completion of the fourth moult (i.e., on the second day of the fifth instar stage). A standard household hand sprayer was used to ensure even distribution of the GSO solution during topical application.

In this study, several key parameters were monitored: larval age during the fifth instar; whole cocoon weight; silk shell weight; pupal weight; and the length and weight of silk filaments obtained from individual cocoons. The age of fifth instar larvae was measured from the onset of the fourth moult to the point when 50% of the larvae had completed cocoon spinning.

Data collected throughout the experimental process were used for comprehensive statistical analysis. This involved exploring patterns within and between treatment groups, summarizing the data into elemental components, and validating predictive models to determine relationships and trends. The core objective of this statistical examination was to identify meaningful variations and trends among the experimental treatments.

Each treatment setup was performed in triplicate to ensure reproducibility and to enhance the reliability of observed results. The statistical parameters considered included the mean, standard deviation, and percentage changes in observed values. Percent variation and Student's *t*-tests were applied to evaluate the statistical significance of differences across treatment groups, using the collected primary data as the foundation for these analyses.

## RESULTS AND DISCUSSION

The age (hours) of fifth-stage silkworm larvae belongs to the group treated with topical application of GSO (through acetone), and the group treated with topical application of linalool (through acetone) was found to record 168.73 (± 13.221) and 177.46 (± 13.786) hours, respectively (Table 1 and Figure 1). The age (hours) of fifth stage silkworm larvae belongs to the group received the leaves of mulberry treated with aqueous solution of Drakshasav and the group received the topical application of acetone solution of GSO followed by feeding with leaves of mulberry treated with aqueous solution of Drakshasav was found recorded 162.87 (± 14.572) and 168.58 (±18.789) hours, respectively (Table 2 and Figure 3).

Tissue-Somatic-Index (TSI) signifies the percentage of tissue in the entire body. Tissue-Somatic-Index (TSI) of the silk glands of silk larvae in the untreated control group; acetone-treated control group and water-treated control group were found recorded as 31.426 units (Table 3 and Figure 2). Tissue-Somatic-Index (TSI) of the silk glands of silkworm larvae in the group treated with topical application of acetone solution of GSO and topical application of acetone solution of Linalool was found recorded 52.625 and 52.728 units, respectively (Table 1 and Figure 4). Tissue-Somatic-Index (TSI) of the silk glands of silkworm larvae, belonging to the group received the leaves of mulberry treated with aqueous solution of Drakshasav, and the group that received the topical application of acetone solution of GSO followed by feeding with leaves of mulberry treated with aqueous solution of Drakshasav, was found recorded 52.759 and 53.854 units, respectively (Table 1 and Figure 5).

 

**Table1.** Characters of the fifth instar larvae of silkworm treated with Vitis-derived herbal juvenoid formulations.

| Parameter Group | Fifth Instar Larval Life Duration (Hours) | Fifth Instar Larval Weight (Gram) | Fifth Instar Silk Gland Weight (Gram) | Tissue Somatic Index of Silk Glands |
|---|---|---|---|---|
| Untreated Control | 145.33(±13.786)00.000 | 03.478(±00.332) | 01.093(±00.107) | 31.426 |
| Acetone Treated (Topical) Control | 145.33(±13.786)00.000 | 03.478 (±00.337) | 01.093(±00.111) | 31.426 |
| Water Treated (Through Mulberry Leaves) Control | 146.59(± 13.003)00.867 | 03.478(±00.339) | 01.093(±00.119) | 31.426 |
| Acetone Solution of Grapeseed Oil Treated (Topical) | 168.73(± 13.221)16.101 | 05.294(±00.569)52.213 | 02.786(±00.213)154.89 | 52.625 |
| Acetone Solution of Linalool Treated (Topical) | 177.46(± 13.786)22.108 | 05.479(±00.623)65.353 | 02.889(±00.339)186.00 | 52.728 |
| Aqueous Solution of Drakshasav (Through Mulberry Leaves) | 162.87(± 14.572)12.069 | 05.563(±00.786)59.948 | 02.935(±00.362)168.52 | 52.759 |
| Acetone Solution of Grapeseed Oil Treated (Topical) followed by Aqueous Solution of Drakshasav (Through Mulberry Leaves) | 168.58(± 18.789)15.998 | 05.786(±00.674)66.359 | 03.116(±00.519)173.01 | 53.854 |

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. *: $P < 0.05$; **: $P < 0.005$; ***: $P < 0.01$

**Table 2.** Characters of cocoon spun by the fifth instar larvae of silkworm treated with Vitis-derived herbal juvenoid formulations.

| Parameter Group | Weight of Whole (with Floss) Cocoon (Gram) | Weight of Whole (Without Floss) Cocoon (Gram) (A) | Weight of Silk Shell of Cocoon (without floss) (Gram) (B) | Silk Shell Ratio [(B÷A) x 100] |
|---|---|---|---|---|
| Untreated Control | 2.962(±0.439)00.000 | 2.873(±0.441)00.000 | 0.558(±0.017)00.000 | 19.422 |
| Acetone Treated (Topical) Control | 2.962(±0.487)00.000 | 2.873(±0.493)00.000 | 0.592(±0.033)00.000 | 19.422 |
| Water Treated (Through Mulberry Leaves) Control | 3.146(±0.831)00.000 | 3.051(±0.557)00.000 | 0.593(±0.041)00.000 | 19.436 |
| Acetone Solution of Grapeseed Oil Treated (Topical) | 5.381(±1.078)81.667 | 5.219(±1.081)81.667 | 1.251(±0.069)124.19 | 23.970 |
| Acetone Solution of Linalool Treated (Topical) | 5.596(±1.788)88.667 | 5.427(±1.149)88.896 | 1.519(±0.347)172.22 | 27.989 |
| Aqueous Solution of Drakshasav (Through Mulberry Leaves) | 5.691(±1.739)92.133 | 5.519(±1.557)92.098 | 1.548(±0.786)176.88 | 28.048 |
| Acetone Solution of Grapeseed Oil Treated (Topical) followed by Aqueous Solution of Drakshasav (Through Mulberry Leaves) | 5.738(±2.013)93.720 | 5.564(±1.786)93.665 | 1.579(±0.998)182.79 | 28.378 |

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. *: $P < 0.05$; **: $P < 0.005$; ***: $P < 0.01$

 

**Table 3.** Characters of silk reeled from the cocoon spun by the fifth instar larvae of silkworm treated with Vitis-derived herbal juvenoid formulations.

| Parameter Group | Silk Filament Length (meter) (C) | Silk Filament Weight (gm) (D) | Denier Scale of Silk Filament [(D ÷ C) x 9000] |
|---|---|---|---|
| Untreated Control | 1173.88(±119.53)00.000 | 0.423(±0.087)00.000 | 3.24300.000 |
| Acetone Treated (Topical) Control | 1173.88(±119.53)00.000 | 0.423(±0.087)00.000 | 3.24300.000 |
| Water Treated (Through Mulberry Leaves) Control | 1171.84(±113.52)00.000 | 0.422(±0.089)00.000 | 3.24100.000 |
| Acetone Solution of Grapeseed Oil Treated (Topical) | 1497.21(±216.64)27.543 | 0.783(±0.123)85.106 | 4.70601.463 |
| Acetone Solution of Linalool Treated (Topical) | 1494.61(±169.55)27.322 | 0.796(±0.118)88.179 | 4.79301.550 |
| Aqueous Solution of Drakshasav (Through Mulberry Leaves) | 1509.55(±173.55)28.594 | 0.819(±0.129)93.617 | 4.88201.639 |
| Acetone Solution of Grapeseed Oil Treated (Topical) followed by Aqueous Solution of Drakshasav (Through Mulberry Leaves) | 1533.28(±352.78)30.616 | 0.843(±0.387)99.290 | 4.94801.705 |

*Note:* 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.

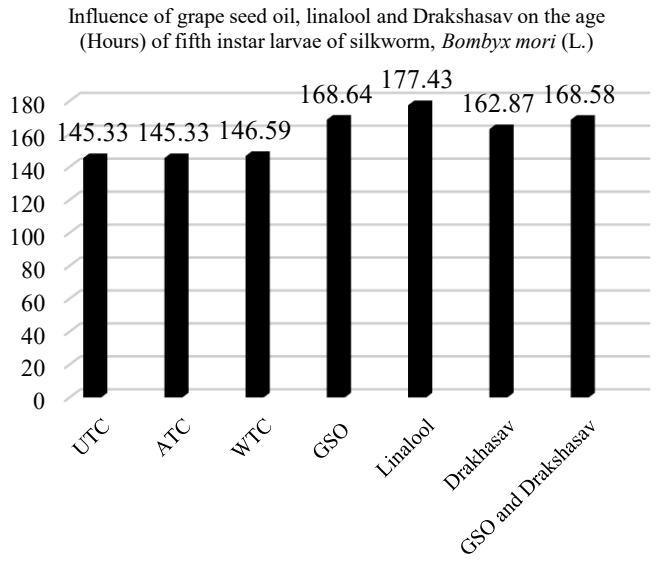


**Figure 1.** Influence of grape seed oil, linalool, and Drakshasav on the age (hours) of fifth instar larvae of silkworm, *Bombyx mori* (L.).

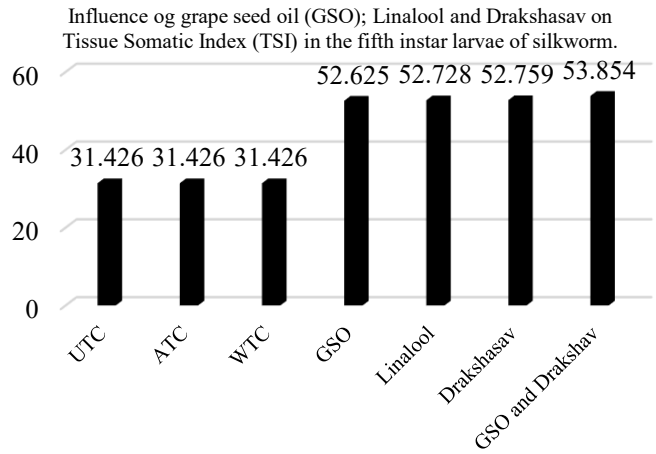


**Figure 2.** Influence of grape seed oil (GSO); linalool and Drakshasav on tissue somatic index (TSI) in the fifth instar larvae of silkworm.

 

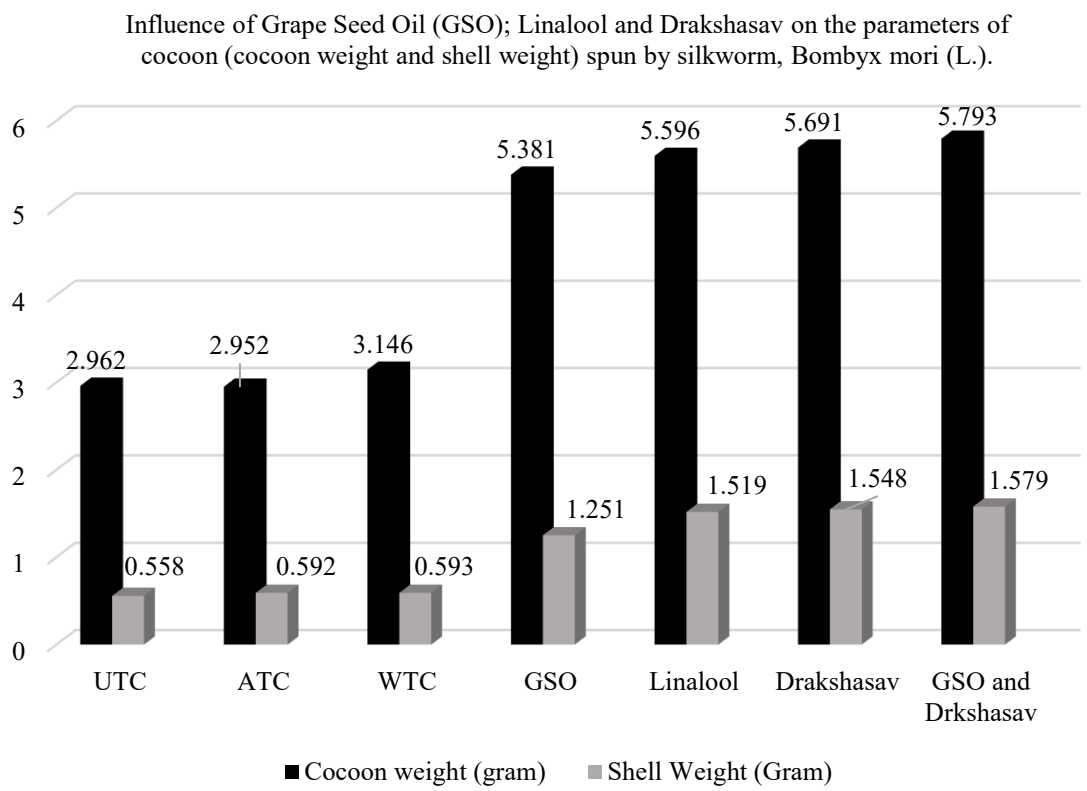


**Figure 3.** Influence of grape seed oil (GSO); linalool and Drakshasav on the parameters of cocoon (cocoon weight and shell weight) spun by silkworm, *Bombyx mori* (L.).

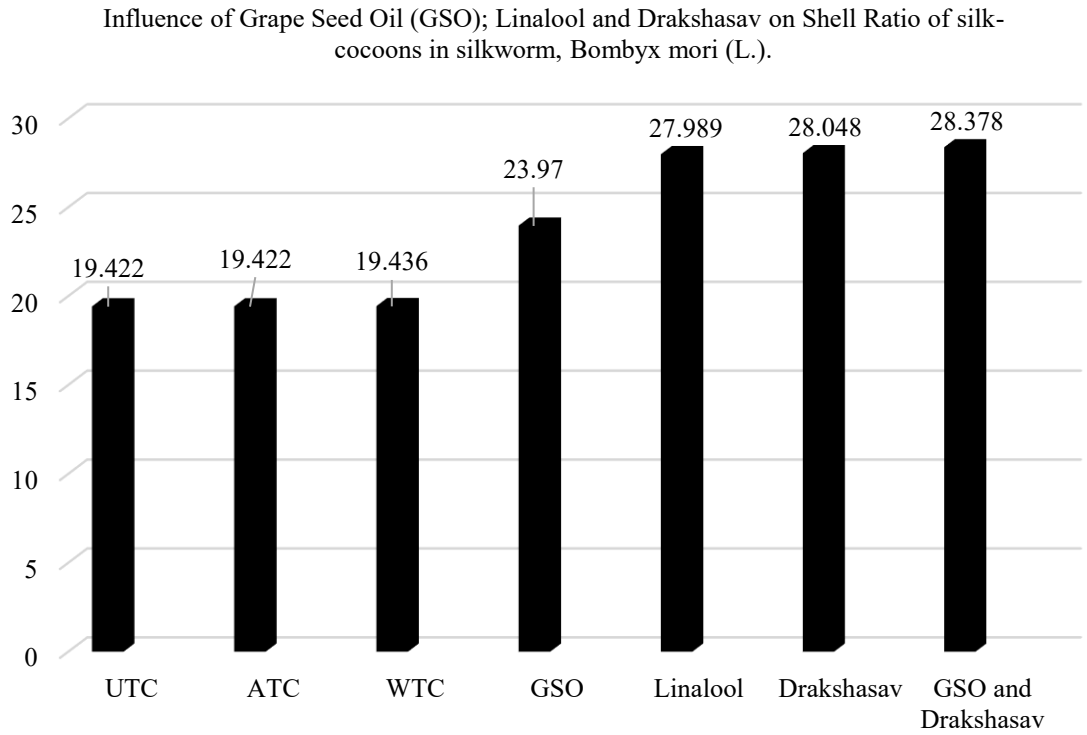


**Figure 4.** Influence of grape seed oil (GSO); linalool and Drakshasav on shell ratio of silk-cocoons in silkworm, *Bombyx mori* (L.).

 

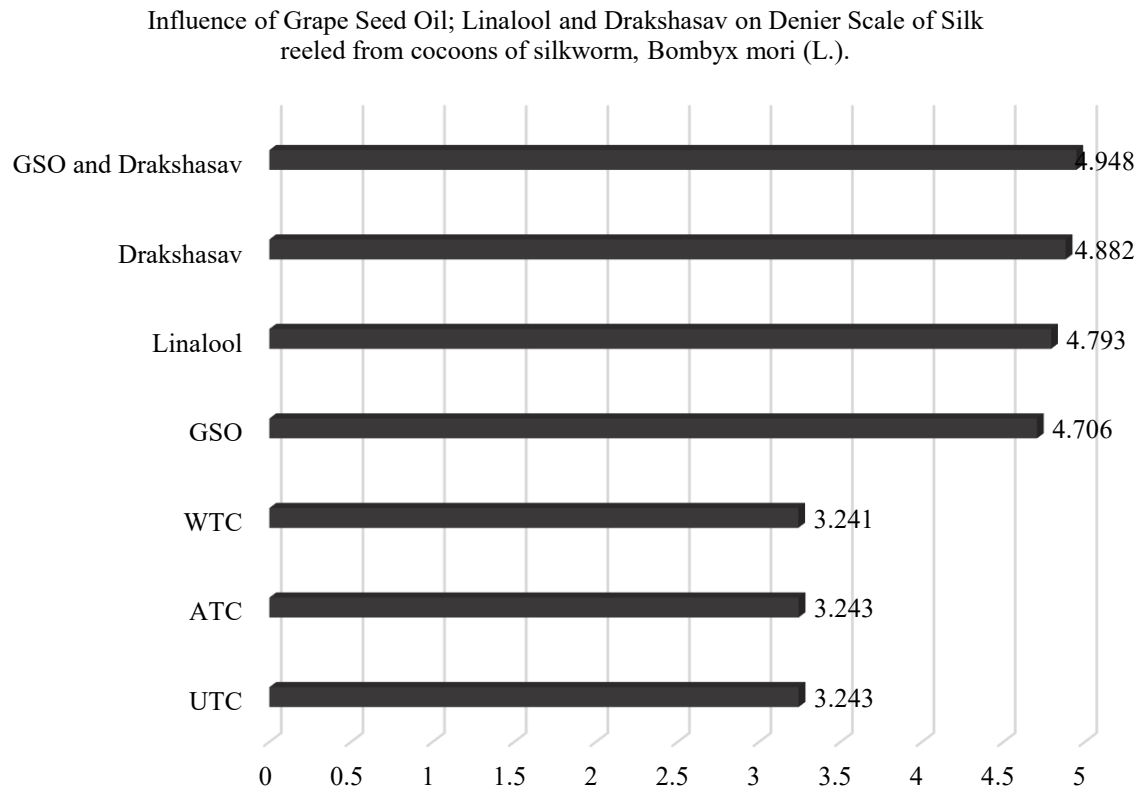


**Figure 5.** Influence of grape seed oil; linalool and Drakshasav on Denier scale of silk reeled from cocoons of silkworm, *Bombyx mori* (L.).

The Denier scale of silk obtained from the cocoons harvested from the group of the group of Linalool Treated group was found to record 4.793 units. The Denier scale of silk obtained from the cocoons harvested from the group of larvae that received the mulberry leaves treated with an aqueous solution of Drakshasav was found recorded 4.882 units. The Denier scale of silk obtained from the group of larvae treated with an acetone solution of GSO, followed by feeding the mulberry leaves treated with aqueous solution of Drakshasav was found recorded 4.948 units.

## CONCLUSIONS

The present investigation clearly demonstrates that both topical and dietary treatments significantly influence the economic traits of *Bombyx mori* during the fifth instar stage. Among the various treatment groups, the combination of topical application of acetone-dissolved GSO and subsequent feeding with Drakshasav-treated mulberry leaves yielded the most promising outcomes in terms of cocoon weight, shell weight, pupal mass, and silk filament characteristics. The consistency of results across triplicate trials reinforces the reliability of the findings. Furthermore, statistical analysis revealed meaningful variations and established the effectiveness of these bio-based interventions in enhancing silk productivity. These observations open avenues for sustainable and nontoxic enhancements in sericulture practices, providing a potential alternative to synthetic growth promoters. Continued research into phytochemical-based treatments may further improve the quality and yield of silk while maintaining ecological and economic balance in sericulture operations.

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recipient of the Nobel Prize of the year: 1998 in Physiology or Medicine for Demonstrating the Signaling properties of Nitric Oxide with Robert F. Furchgott and Ferid Murad). With the best compliments from India, the present attempt on "Utilization of Herbal-Juvenoid-Formulations and Herbal Juvenoid Compound for Qualitative Silk Cocoons from Silkworm, *Bombyx mori* L" wishes happy birthday to Hon. Louis Joseph Ignarro.

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