**A comparative study to evaluate the efficiency of Coleus forskohlii root extract and Forskolin as a repellent against Uzi fly (Exorista bombycis)**

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**Abstract:** Coleus forskohlii (CF) is a well-known medicinally important plant and it has been part of Ayurveda for decades. Forskolin, a diterpene and active constituent of Coleus forskohlii root extract (CFRE). The CFRE has been used for treating various infections and chronic diseases. But the CFRE has never been used as a pesticide. Exorista bombycis (Uzi fly) is a common pest found to infest Bombyx mori (Silk worm) and cause severe loss to sericulture farmers or community. Uzi fly targets the fifth instar larvae of silk worm to lay its eggs and for the propagation of its species. CFRE and Forskolin were evaluated for its anti-Uzi fly properties and the Uzi fly maggots, Uzi fly pupae were treated with different concentrations of CFRE and Forskolin were used i.e. 0.5, 1.0, 1.5, 2.0 μg/μL. It was observed that the normal growth and development of Uzi fly were hindered at different stages of its life cycle. The fifth instar larvae of silkworm were treated with 0.5-2.0 μL of CFRE and Forskolin which resulted in oviposition deterrence, reduced egg hatching, aberrations and deformities in pupae and adult (i.e. molting process, ecdysial failure) and adult emergence from pupae. The observations from the present study clearly suggest that CFRE and Forskolin can be an alternative for commercially available pesticides for preventing the propagation of Uzi fly by influencing its growth, development, and metamorphosis of immature stages of Uzi fly. The efficacy of CFRE is slightly better than Forskolin in exerting its anti-Uzi fly properties.

**Key words:** Coleus forskohlii; Forskolin; Exorista bombycis; Morphogenic deformities; Growth-inhibition

**Introduction**

Sericulture, an agro-based industry is aiding in the livelihood of many farmers throughout India and in particular in South India. A new threat was encountered during 1980 in the form of a pest which was later recognized as Uzi fly [1]. Silkworm cultivation in India as well as other silk producing countries has been very much affected by the Uzi fly (Exorista sorbillans). It is a hyper parasitoid on silkworm, Bombyx mori L. [2]. Despite adopting several preventive measures, Uzi pestilence recorded 9 to 40% loss in the cocoon production [3]. An integrated pest management concept has been recommended to control this pest which includes cultural/mechanical, exclusion, chemical and biological methods[4]. Various measures were suggested by several researchers for annihilating the infested silkworms using fly proof wire meshes and nylon nets to prevent their ingress, spraying of uziicide, usage of uzi-traps etc., [5-7].

Sericulturalists have adopted all these methods in their routine practice for pest control. Most pesticides even in minute doses with a distinct course of action altered insect reproduction. The attributes like the quality of cocoon, eclosion, and fecundity, growth and reproduction of the silkworm are greatly influenced by the insecticides [8]. Use of
synthetic chemicals to manage these pests resulted in their revival and onset, resilience to insecticides, elimination the existing natural antagonists additional to polluting the environment. Consequently, the quest for contingent and rational approaches to synthetic pesticides is in succession [9-10]. Among them, novel natural substances derived from higher plants are preferred over others due to its environmental safety [11]. Plant derived products are preferred as suitable alternatives to harmful synthetic pesticides. Plant products could stimulate diverse modes of deformities in pests, which could safely be used in pest control [12].

*Coleus forskohlii* Wild is a medicinal plant used since prehistoric times for the treatment of diseases mainly affecting heart, abdomen and lungs. The root anatomy of *C. forskohlii* holds a thick, fibrous, radially spread, golden brown organization. Their size is 20 cm long and 0.5–2.5 cm in diameter. The stem has four-angled branches and hair-like nodes [13]. The *Coleus forskohlii* root extract has a comprehensive phytochemical constituent deactyl forskolin, 1, 9-deoxyforskolin, forsko diterpeneside C, D, and E, labdane diterpeneglycosides, labdane diterpene forsko diterpe A, 1, 9-dideoxy-7-deacetyl forskolin (7 acetoxy 8, 13-epoxy-1, 6, 9-trihydroxylabd-14-en-11-one)[14-15]. Forskolin consorts with various physiological processes of insects, affecting the morphological characters as well as growth and development. *Coleus forskohlii* root extract diminishes the oviposition rate and suppress the adult emergence of uzi fly [16]. Forskolin displays a variety of enzyme-inducing, anti-inflammatory, immunomodulating, antimicrobial and as well as anti-leukemia and cancer [17].

**Life cycle of Uzi fly:**
The *E. bombycis* is a holometabolous insect The Uzi fly (*Exorista sorbillans*) life-cycle involves four stages- egg, maggot, pupa and adult (Fig.1). Most of the uzi fly will emerge into maggots from the pupa within 11 -12 days in the silkworm rearing trays. These adult male and female flies mate within 12 - 18h after emergence and the copulation lasts for two to four hours.

**Life cycle of Exorista bombycic (Uzi fly)**

![Life cycle of Exorista bombycic (Uzi fly)](image_url)

1. Uzi Fly
2. Adults depositing eggs on silkworm
3. An egg on host body
4. Black scar on silkworm body
5. Maggot
6. Pupa
7. Adult Uzi fly

**Figure 1:** Life cycle of the uzi fly, *E. bombycis* showing all the four stages viz., egg, maggot, pupa, and adult: Newly emerged *E. bombycis* flies were mated and gravid mated females ovipositioned within 24h. The flies laid eggs on day 1 of fifth instar larva of *B. mori*, hatched after 48h, invaded the silkworm, completed maggot period inside the silkworm as an endoparasite, exited out of silkworm, pupated outside and transformed to adult flies within 11 days. Note the cuticular lysis at the site of infection. Melanization of the maggot is seen from outside through translucent cuticle (arrow). Maggot showing melanization and partial encapsulation were dissected out from the infected *B. mori* larva at 72h after infection.
In our present study, an effort has been made to understand the effect of *Coleus forskohlii* root extract (CFRE) and Forskolin, a diterpene (i.e. an active compound obtained from the roots of *Coleus forskohlii*) on Uzi fly to provide an environmentally safe and a profitable road to farmers against Uzi fly pestilence.

**Materials and Methods**

*Coleus forskohlii* root powder was purchased from local markets in Hyderabad, Telangana. Forskolin and Acetone were procured from Sigma Aldrich, USA. Conical flasks, Capillary tubes, and sprayer were purchased from SR Lifesciences, Hyderabad, Telangana. Mulberry leaves, infected instar larvae of *Bombyx mori* L., infested *B. mori* cocoons, maggots of Uzi fly were procured from markets in Khadiri, Ananthapur, Andhra Pradesh.

Preparation of *C. forskohlii* root extract (CFRE) and forskolin solution

10 mg of *Coleus forskohlii* root powder was dissolved in 10 mL of absolute acetone and the mixture was vortexed for 5-10 minutes for dissolving the mixture and strained using fine sieves and stored in an amber-colored bottle until further use. Similarly, 1mg of commercially available Forskolin was dissolved in 1mL of absolute acetone and the mixture was vortexed for 5-10 minutes for dissolving the mixture and strained using fine sieves and stored in an amber-colored bottle until further use.

Collection and rearing of Uzi fly

The Uzi fly fresh maggots were collected immediately after they pierced out from their host body through cocoon shell are allowed to pupate in dark by keeping the maggots in an enamel tray (6” x 9”) covered with black paper. The maggots were transformed to pupae within hours. After 5-6 days, the pupae were transferred to the wire mesh cages of 24” x 24” x 24” dimensions. The emerged Uzi flies were fed with honey, soaked in cotton balls. The flies were allowed to mate freely for 24 - 48 hours. Fifth instar silkworm larvae were utilized for studying the ovarian deformities and oviposition deterrence in Uzi fly.

Treatments

The *C. forskohlii* root extract (CRFE) and Forskolin were tested on different stages of Uzi fly life cycle in order to evaluate their efficacy in controlling the morphogenesis or development of maggots into adult Uzi fly. So, we used to different concentrations (0.5, 1, 1.5 and 2.0 μL) of *C. forskohlii* root extract and Forskolin was prepared and used in the present study.

Statistics

The observed results were subjected to One-way ANOVA and T-test for calculating the significance of treatments using SPSS software version 24. Statistically the p-value of <0.05* was considered significant, whereas p values of <0.01** were considered very significant and <0.001*** was considered as highly significant.

Results

Figure 2, illustrates the effect of CFRE on Uzi fly propagation. On treatment with CFRE and Forskolin, there was significant reduction in Egg hatching’s, morphogenesis of Uzi fly maggots in to pupa and adult, fecundity and oviposition deterancy in maggots, pupae and adults when treated with different concentrations of CFRE and Forskolin (0.5, 1.0, 1.5, and 2.0 μg/ μL). The Uzi fly maggots were unable to develop properly into health adult, as these treatments were causing aberrations/deformities in their pupal intermediates, adults leading to their death.

The figure 2, illustrates the effect of Forskolin on Uzi fly propagation. On treatment with CFRE and Forskolin, there was significant reduction in Egg hatching’s, morphogenesis of Uzi fly maggots in to pupa and adult, fecundity and oviposition deterancy in maggots, pupae and adults when treated with different concentrations of Forskolin (0.5, 1.0, 1.5, and 2.0 μg/ μL). The Uzi fly maggots were unable to develop properly into health adult, as these treatments were causing aberrations/deformities in their pupal intermediates, adults leading to their death.
Figure 2: A). Effect of CFRE on Uzi fly egg hatching's; B). Effect of CFRE on Uzi fly maggots; C). Effect of CFRE on zero-hour pupae of Uzi fly; D). Fecundity in resultant adults from maggots after CFRE treatment; E). Effect of CFRE on oviposition deterrence of Uzi fly.

Figure 3: A). Effect of Forskolin on Uzi fly egg hatching's; B). Effect of Forskolin on Uzi fly maggots; C). Effect of Forskolin on zero-hour pupae of Uzi fly; D). Fecundity in resultant adults from maggots after Forskolin treatment; E). Effect of Forskolin on oviposition deterrence of Uzi fly
Discussion

Forskolin which is produced by roots of the plant Coleus forskohlii is chemically a labdane diterpene. This compound displays a variety of pharmacological activities disposing Anti-microbial and anti-inflammatory. It also regulates cell mediated immunity functions, as well as cytotoxic effects against leukemic and human tumor cell line. Forskolin mode of action is by stimulating the adenylate cyclase enzyme, which leads to an increase in the most important cell-regulating compound cyclic adenosine monophosphate (cAMP)[18]: It excites the cell receptors by activating the enzyme adenylyl cyclase and increasing the intracellular levels of cyclic Adenosine Mono Phosphate (cyclic AMP)[19-20]

The results of the present study demonstrate that the oviposition response of E. bombycis was influenced greatly on CFRE treated silkworms compared to the control. Among the concentrations i.e., 0.5 μL, 1 μL, 1.5 μL and 2.0 μL of forskolin extract used, at 2.0 μL Uzi flies exhibited maximum oviposition deterreny, fecundity, egg hatching’s (Figure 2). However, higher the concentration of forskolin extract greater was the reduction in oviposition of Uzi fly. CFRE could be an effective alternative against adult Uzi fly to reduce the oviposition on the treated fifth instar silkworm larvae. This action could be the result susceptibility of Uzi fly to CFRE there by impede egg laying on the body of silkworm larvae. Similar studies were reported by Dr. Cheruku (2017) using neem oil on oviposition deterrence of Uzi fly, Mansour et al., (1986) working on Tetranychus cinnabarinus and by Dhar et al., (1996) with neem against Anopheles stephensi. [21-25]

Effect of CFRE and Forskolin on maggots, zero-hour pupae of Uzi fly was 73.33%, of the treated pupae emerged into adults which were morphologically deviant with shunted growth. They had asymmetric bodies, incomplete metamorphosis of the abdomen with abnormal wings. CFRE and Forskolin caused moult disruption, formation of permanent larvae, larval-pupal intermediates, pupal-adult intermediates, deformed, non-viable pupae and abnormal adults (Figure 2-3). Forskolin can prevent or reduce progeny production by influencing growth and development of immature stages. Similar studies were reported by Sukumar et al., and Lingampally etal in their studies. [24-25]

Conclusion

The present study suggests the usage of CFRE and forskolin for efficient uzi fly pestilence at larval stages. Forskolin can materialize as an effective growth and development inhibitor in Integrated pest management modalities. According to the results of this study and other reports, the application of CFRE on the Uzi fly induces morphological deformities. The plant extract has influenced the sustainability of the pests by hindering the growth and development suggesting its use as an insect growth regulator in pest control. It is concluded that instead of using synthetic insecticides for pest control in sericulture, CFRE can be recommended as these medicinal plant extracts are found to only interfere with the life cycle of the parasitoid without affecting the host i.e., silkworm- Bombyx mori. Further investigation is needed to help us to deeply understand the mechanism involved at the molecular level both in plant extract and larvae also.

Acknowledgement’s

I would to extend my thanks to my guide Prof. Sabitha S. Raju and my colleagues from Sarojini Naidu Vanita Maha Vidhyala, Hyderabad.

References


Cite this article as:

Source of support: Nil; Conflict of interest: Nil.