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Antibacterial Activity of Clove Oil Extracted from Eugenia Caryophyllaea and its Effect on the Economic Traits of Mulberry Silkworm *Bombyxmori, L.*

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Abstract
The significant antibacterial activity of the clove oil was determined against Bacillus cereus and Proteus vulgaris hence the oil has been proved as a natural antimicrobial agent. The major components in the oil are acetyl eugenol, beta-caryophyllene, triterpenoids like oleonolic acid, stigmasterol and campestral. Mulberry silkworm is an economically important insect, its economic traits improved after feeding with mulberry leaves fortified with clove oil of four different concentrations. The commercial characters increased at T_3 treatment. Application of clove oil in sericulture farming may boost the larval growth subsequently the commercial characters.

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INTRODUCTION

Man took advantage of medicinal and aromatic plants since ancient times to control many diseases hence plants are warehouses of phytochemicals and secondary metabolites that have antibacterial, antifungal and antiviral properties. Aromatic plants synthesize and preserve secondary metabolites which are referred as natural biochemical factories or chemical gold mines. The pharmacological activity of these aromatic plants are classified into four acceptable groups namely alkaloids, glycosides, essential oils and other substances on diagnostic basis (Baby P. Sakaria. 2007). Large number of antimicrobial agents tapped from higher plants are used to combat wild variety of microbial infections. Globally, a good source of raw materials for these antimicrobial agents are the flora and fauna which continue to play a dominant role for combating various serious diseases with their phytochemical active substances that produce diversified physiological action on beneficial organisms including silkworm (Cragg G.M. et al., 1999). Essential oils are defined as volatile oils extracted from aromatic plants having wild and varied bioactivity against insects and they are commonly referred to as volatile plant secondary metabolites. Hence, knowledge of these secondary metabolites is essential.

The essential oils are extracted from plant families that include Asteraceae, Labiaceae, Myrtaceae, Rosaceae, Rutaceae, Umbelliferae, Lauraceae etc. Essential oils are optically active, non-greasy, partially soluble in water but fully in alcohol and carrier oils. Chemically free and harmless ingredients have been used immemorial as an alternate remedy for treatment of many infectious diseases (Reynolds, 1998). The constituents of the oil are monoterpene, sesquiterpens hydrocarbons with general formulae (C_5H_8) n. The activity of those of essential oils can be compared with the activity of these synthetically prepared essential oils. These are extracted by distillation (hydro distillation, hydro steam distillation and steam distillation), maceration, effleurage, extracted with volatile solvents and super critical fluid extraction.

Sericulture plays an important role in antipoverty programme, prevents migration of people, the occupation emerges as an alternative income source for unprivileged rural people thus helps in improving their quality of life, ultimately benefits their economic growth and development of family.

The success of silk industry depends on protection and management of silkworm crops from four common silkworm diseases, of these, bacterial flacherie is a serious disease that is being suppressed by antibiotics like Erythromycin, Streptomycine, Terramycinetc, prolong exposure to these might lead to development of resistance in silkworms on long run. Hence in this present study, we have utilized few essential oils of medicinal value extracted from aromatic plants against these bacteria isolated from the gut tissue of *Bombyxmori*. With passing of time, it was found that a wild variety of essential oils from aromatic plants are not only used as fragrance or flavors but also for their antibacterial activity. Hence, attempt has been made to study the effect of essential oils / plants extracts of one / few plants against few organisms by using protocols like disc diffusion, pour well, swab disc etc, as means of determining minimum inhibition zones (Kuntamallaand Sammaiah, 2007 and Charles and Simon, 1998; Eloff, 1998).

In the last decade, review about supplementing and additives to mulberry silkworm have been studied (Eid et al., 1989, Priyanka et al., 2014), in the process, this work has also been aimed to study the effect of these aromatic plants on silkworm performances. As these plants based secondary metabolites showed phagostimulant and growth promoting effect on silkworms thus leading to improvement in larval and commercial characters when fed with mulberry leaves fortified with these essential oils (Shahin et al., 2013; Dubey and Srivastava et al., 2014; Tiwari et al., 2014).

MATERIALS AND METHODS

Clove oil extracted from *Eugenia caryophyllaea* was used for fortification.

Botanical description of plants:
Eugenia caryophyllaea is an ever green plant that grows to a height of 8 to 12m with large leaves, the flower bud is initially pale green color but at the time of harvest when extracted from the clove it comprises 72 to 90% of the essential oils. The other constituents of clove oil are acetyl eugenol, beta-caryophyllene, triterpenoids such as oleanolicacid, stigmasterol and compesterol.

Vernacular names:
Telugu – Lavangamu
Tamil -Ainnilam, Ancukam
Sanskrit – Lavangalata, Dhanshika

In vitro studies on the effect of these essential oils:
Multivoltine rearing of Kola gold was reared as per the standard rearing method suggested by (Dandin et al., 2002) using V1 variety. Silkworm cadavers affected from flacherie were selected for experimental studies (Poinar et al., 1984). Essential oils of different concentrations (1:1, 1:5, 1:10 and 1:20) were prepared and added to the nutrient agar media. A loop of bacterial cultures drawn from the cultures of *Bacillus cereus* (Grampositive) and *Proteus vulgaris* (Gramnegative) were streaked on the plate and kept for incubation. Observations were made on the growth after 48 hours.

Mode of application of test solution:
Early 4th instar silkworms 24 hours of 3rdmoult were selected for the treatment. The larva were counted grouped into 18 batches (100 larva each), which includes the test and control batches. The four different concentrations of oil i.e, 1%, 5%, 10% and 20% were prepared to serve as treatment along with control (Methanol) and normal (water) batches. The test insects were treated by fortification method using atomizer for fortifying the mulberry leaves. The treatment was replicated thrice. Different concentrations of oils were supplemented once in 4th instar and daily in the 5th and fed to the silkworms. Fresh leaves dipped in fresh water were used as normal. In the study, an attempt has been made to screen the antibacterial activity of the clove oil against bacteria *Bacillus cereus* and *Proteus vulgaris* isolated from the gut tissue of silkworm on basis of the diameter of the inhibition zone and to study the effect of these oils on economic parameters of silkworm.

RESULTS

Clove oil showed activity against gram positive bacteria while it showed greater activity against gram negative. Clove oil showed an inhibition zone of 6mm against both strains at 1:5 concentrations. While at 1:1 ratio, it showed an inhibition zone of 6mm against both *Bacillus cereus* and *Proteus vulgaris*.

Table 1: Effect of essential oil on *Bacillus cereus* and *Proteus vulgaris* with four concentrations (1:1, 1:5, 1:10, 1:20)

Sl.No.	Essential oil concentration	*Bacillus cereus*	*Proteus vulgaris*
1	1:1	6	6
2	1:5	3	3
3	1:10	3	2
4	1:20	3	2

Each value is mean of five observations

Effect of clove oil on larval weight of silkworm:
When clove oil was fortified on mulberry leaves and fed to silkworms as a feed it showed a positive effect on the larval weight with increase in concentration. In the 3rd instar, there was a gradual increase in the larval weight from T_1 to T_3. In T_4 it gradually decreased to 4.51 gm. During 4th instar maximum larval weight of 10.05 gm/10 worms for observed as 1:10 ratio followed by 1:5, 1:1 and then by control. When 5th instar larvae were taken into consideration it was found that in the middle of 5th instar, maximum larval weight was found in 1:1 concentration batch while at the time of mounting among all the concentrations on par with control and normal batches, maximum weight of 38.00 gm/10 worms was found at T_3 treatment followed by T_2 (37gm) and minimum was found in the control.

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Table 2: Effect of clove oil larval weight of mulberry silkworm *B.mori, L*

Sl No	Treatment	I Instar (gms)		II Instar (gms)		III Instar (gms)		IV Instar (gms)		V Instar					
										At beginning Max weight (gms)		In the Middle Max weight (gms)		At the time of mounting Max weight (gms)	
		Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE
1	**T1**	0.24	0.01 ±0.01	0.59	0.04 ±0.02	4.26	0.21 ±0.10	9.31	0.01 ±0.01	18.22	0.36 ±0.18	27.15	0.02 ±0.01	36.61	0.34 ±0.17
2	**T2**	0.25	0.01 ±0.01	0.59	0.25 ±0.12	4.92	0.17 ±0.09	9.45	0.01 ±0.01	18.75	0.14 ±0.07	32.75	0.03 ±0.01	37.00	0.36 ±0.16
3	**T3**	0.27	0.02 ±0.01	0.60	0.13 ±0.06	5.04	0.29 ±0.15	10.05	0.01 ±0.01	19.22	0.25 ±0.13	33.75	0.02 ±0.01	38.00	0.04 ±0.02
4	**T4**	0.25	0.02 ±0.01	0.58	0.17 ±0.09	4.51	0.17 ±0.09	9.21	0.02 ±0.01	17.89	0.21 ±0.10	31.12	0.07 ±0.04	36.00	0.31 ±0.16
5	**Control**	0.23	0.01 ±0.01	0.51	0.23 ±0.12	3.84	0.10 ±0.05	8.75	0.15 ±0.08	13.80	0.11 ±0.05	24.21	0.03 ±0.01	31.30	0.00 ±0.00
6	**Normal**	0.23	0.10 ±0.5	0.54	0.26 ±0.13	3.92	0.32 ±0.16	8.66	0.02 ±0.01	13.60	0.18 ±0.09	22.93	0.01 ±0.05	30.62	0.02 ±0.01

Each value is the mean of 5 observations. SD and SE are standard deviations and standard error.

Table 3: Effect of clove oil on economic characteristics of silkworm *B.mori,* L.

Sl. No	Treatment	Average Cocoon wt. (gms)		Average Pupal weight (gms)		Average Shell wt (gms)		Average shell ratio (%)		Average filament length (m)		Denier		Error (%)	
		Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE	Mean	SD/SE
1	**T1**	1.22	0.0153 ±0.0076	1.01	0.0058 ±0.0029	0.20	0.0023 ±0.0012	17.22	0.1976 ±0.0988	783.05	45.30 ±22.65	22.80	0.06 ±0.03	95.54	0.159 ±0.721
2	**T2**	1.27	0.125 ±0.0625	1.06	0.0981 ±0.0491	0.21	0.0313 ±0.0156	20.49	0.79 ±0.395	819.93	27.37 ±13.69	21.85	0.81 ±0.41	96.53	0.0160 ±0.627
3	**T3**	1.29	0.03 ±0.015	1.06	0.0306 ±0.0153	0.23	0.0036 ±0.0018	21.49	0.6947 ±0.3474	842.30	24.50 ±12.25	18.98	0.15 ±0.08	97.52	0.0173 ±0.362
****4**	**T4**	1.22	0.1 ±0.005	1.02	0.0208 ±0.0104	0.20	0.02 ±0.01	19.32	1.005 ±0.5025	698.30	15.45 ±7.84	20.50	0.15 ±0.076	95.16	0.0793 ±0.535
5	**Control**	1.13	0.0379 ±0.0189	0.93	0.0252 ±0.0126	0.20	0.004 ±0.002	17.13	0.1358 ±0.0679	666.83	6.70 ±3.35	22.93	0.49 ±0.25	94.20	0.0621 ±0.347
6	**Normal**	1.00	0.1069 ±0.535	0.80	0.0896 ±0.0448	0.20	0.011 ±0.0055	16.91	0.79 ±0.395	663.65	16.79 ±8.40	22.84	0.26 ±0.13	94.12	0.0891 ±0.414

Each value is the mean of 5 observations. SD and SE are standard deviations and standard error.

Effect of clove oil on commercial characters of silkworm *Bombyxmori*:

The observation recorded on effect of feeding of different concentrations on clove oil on the commercial characters of mulberry silkworm is presented in table3.Cocoon and shell weights increased in clove oil treated batches when compared with control and normal batches. Among the treatments, T_3 showed significant increase in average cocoon weight and shell weight, while other treatments showed a marginal increase in cocoon weights. Significant increase in pupal weight was noticed in T_3 batch when compared to control and other treatments. Increase in average shell weight was noticed in treated batches in comparison with control. Maximum SR% was noticed in T_3 (21.49$\pm$ .79) followed by T_2 (20.49 $\pm$ 69) batch, silk filament length was highest (842.3m $\pm$ 27.37) in T_3, followed by T_2 (819.9m$\pm$ 45.3), T_1 (783.9m $\pm$ 24.5), T_4 (698.30m $\pm$ 15.45) and control (666.83m $\pm$ 16.2).

DISCUSSION

It is found that at all dosage levels the oil did not show any lethal symptoms of poisoning such as restlessness, contraction of the body, ejection of gastric juices from the mouth and skin folds.

The increase in larval weight with increase in concentration is due to enhancement of bio availability of nutrients for digestion with these essential oils resulting in the growth of the silkworms. It can also be related to growth promoting effect of the essential oils due to the stimulation of protein synthesis, nucleic acid and other macro molecules (Fatima et al., 2014).

Feeding preferences of the insects are mediated by presence and absence of primary and secondary metabolites. Silkworms utilize only few compounds from their food plants as feeding stimulants. As per the response of the silkworm larva the chemical stimuli are attractance, bitingand swallowing factors.

According to Hamamura et al., (1961) silkworms are attracted by three stimulants. He reported that βsistosterol is responsible for stimulating larval biting action, where as the terpenes compounds linalool and citral act as attractants for silkworm. The components present in clove oil are acetyl eugenol, tannin, flavonoids, triterpenoids and olieonolic acids havestrong growth promoting action on mulberry silkworms.

It might be found that the physiological stimulation by this clove oil on the silkworm larva might have lead to maximum larval growth due to consumption of more food and thereby increase in economic parameters (Alagumani Kumaran et al., 2016).

On basis of the present observations and above information it can be concluded that the increase in economical parameters may be due to control of microbial pathogens causing silkworm diseases there by increased rate of metabolism due to enhanced feeding behavior resulting in maximization of commercial characters.

CONCLUSION

Based on the above investigation, it can be concluded that the natural oils of *Eugenia caryophyllaea* with its antibacterial activity, enhanced growth of mulberry silkworms are likely to throw light on the possibility of using the oil as natural antibacterial agents against the two bacterial strains isolated from silkworms and also as a food additive to promote silkworm growth with enhanced silk productivity showing no negative effect. Through this study, it would also be useful to gain insight into the efficiency of a safe disease management in sericulture with enhancement of commercial characters with use of this essential oil.

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