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Antibacterial Activity of Phyto Essential Oils on Flaccherie Causing Bacteria in the Mulberry Silk Worm, B.Mori. L

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Abstract: *The study was designed to examine the antimicrobial activity of eight essential oils of different concentrations against bacterial species by using disc diffusion technique on nutrient agar media, based on their inhibition zones. The activity of the essential oils was evaluated against Bacillus cereus and Proteus vulgaris which are common pathogens attacking silkworms in our area. The results indicate that all essential oils exhibit antibacterial activity against the silkworm pathogens.*
Out of the eight oils, cinnamon (8mm), clove oils showed maximum inhibitory effect against gram +ve bacteria while lavender and cinnamon oil followed by other oils against gram – ve bacterial strains.
Key Words: *Essential oils, Flaccherie, mulberry silkworm, antibacterial activity.*

I. Introduction

The problems with drug resistant micro-organisms is the emergence of new diseases with no proper medication,the side effects of modern drugs have stimulated renewed interest in plants as source of new medicines[1].In recent years evoke interest in use of phytochemicals as antimicrobial agents has resulted in thorough investigation of medicinal plants[2].In Western medicine, substances derived from plants constituted 25% of prescribed medicines and 74% of the 121 bio active plant derived compounds are identified via research[3].

Aromatic plants and their essential oils used as food preservatives are known sources of anti-microbial action and their preparations have application in field of phytopathology and pharmacy.Essential oils are natural, composed mainly of terpenesas well as other non terpene components that can be considered in the future for more biological applications in current medication. An estimated 3000 essential oils are identified of which only 300 are commercially marketed[4]. Essential oils are good source of biologically active compounds, thus it is reasonable to expect these compounds to have antimicrobial activity.Essential oils (volatile oils) are aromatic, oil liquids extracted from plants by hydro distillation.

Indian sericulture is an important agro based industry because of its employment potential, entrepreneurship opportunities, stable and high income. But, frequent out break of diseases lead to crop failure which are major constrains in sericulture.Hence, researchers are striving hard to minimize the disease incidence and maximize cocoon production. Among the survey reports[5], the incidence of bacterial flaccherie is highest (57.2%), followed by cytoplasmic polyhedrosis virus (27.88%), nuclear poly hedrosis (5.0%),pebrine (2.32%) and muscardine (0.48%). The disease occurrence can be prevented / controlled by using different chemical disinfectants, however some of the ingredients used in these formulars are causing environmental pollution, health hazards to both insect and humans. As scientific literature on antibacterial activity of essential oil against silk worm diseases is very scanty hence, the study was undertaken to report the antimicrobial activity of EO isolated from aromatic plants and also to investigated whether their oils could contribute to access new antimicrobial products with pharmacological and industrial application in silk industry.

II. Materials and Methods

The eight essential oils were selected for the study based on literature survey and their use in traditional medicines.

Source for collecting silkworm:

The biviltineseggs were procured from Vijayawada grainage Andhra Pradesh. The silkworms were reared in rearing house (Kakatiya University) by following the standard rearing technique as recommended[8]. Regular examination was made to collect the silkworm cadavers in sterilized vials based on the symptoms and kept in the refrigerator at 4^{o}C for further use.

Isolation of bacterial pathogens from gut tissue:

Silkworm cadavers showing bacterial infection were surface sterilized with 0.1% mercuric chlorideand then washed with sterile distilled water no of times. The bacteria that were isolated from the gut were streaked

on the nutrient agar medium, incubated at room temperature. Using streak plate technique, the bacterial colonies were further purified, after attaining good growth; slants were stored in refrigerator at $4^{O}C$ for further studies[9] and used as stock cultures. The bacterial pathogens of silkworm were identified based on morphological characteristics such as colony morphology and staining techniques.

Anti-bacterial Assay:

Screening of eight essential oils for antibacterial activity was done by disc diffusion methodbased on inhibition zone of the bacteria[10].

III. Results

Eight essential oils were evaluated for antimicrobial activity against pathogenic strains of gram positive (Bacillus cereus) and gram negative (Proteus vulgaris) bacteria. The oils were found to be active against all bacterial strains with different inhibition zones ranging from 1-8mm. Against the gram positive bacteria, lavender, cinnamon,cloveand rose oil showed activity while against gram negative, clove and cinnamon oil showed high activity,some essential oils at lower ratios (1:20 and 1:10) showed high antibacterial activity than higher concentrations(1:5 and 1:1).Highest inhibition zone (8mm) at 1:10 was obtained with lavender,cinnamon oil against B. cereus (gram positive) while lowest was observed with orange, olive, clove and lemon grass ratio at 1:20 ratio Cinnamon oil at 1:10 concentration showed highest inhibition zone (6mm) against P.vulgaris (gram negative).Clove oil showed an inhibition zone of 6mm against both strains at 1:5 ratio concentration.

IV. Discussion

There is a growing interest on essential oils of plants as an alternative remedy for disease treatment. Numerous researchers showed increased interest on these oils which are rich source of biologically active compound which developed resistance against antibiotics a common phenomenon on long term use of synthetic drugs.[11]

The highest antimicrobial activity was observed on gram^{+}positive bacteria as opposed to gram negative bacteria because they posses outer membrane surrounding the cell membrane which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering[12]. In the studies, lavender oil showed high antibacterial activity on par with cinnamon oil at 1:10 concentration which suggests that antimicrobial activity is due to major and minor components (Linaloo, linelylacetate, Eucalyptol and borneol in lavender oil[13] and the essential oil extracted from cinnamon sps showed high inhibitory activity in this study which could be due to cinnamon dehyde (37.6%), cinnamyl acetate (23.7%) and cinnamyl benzoate (16.4%) which possesses polar hydrophilic functions (OH, COOH, NH_2, NO_2)[14].

The studies on antibacterial activity of clove oil showed highest zone of inhibition against gram positive bacteria which is due to the content of eugenol, which is a monoterpene, in the range of 47.67% to 78% obtained from Syzigiumaromaticum (Fam – Myrtaceae)[15,16].

V. Conclusion

From this study it can be concluded that the eight essential oils possess antibacterial activity against bacteria strain isolated from gut tissue of silkworm B.mori,L. Among the oils isolated,lavender and cinnamon oils showed high antibacterial activity against silkworm bacterial strains and further evaluation of the potentiality of these oil as an antibacterial agent either as topical or oral applications is need of an hour for silk industry.

Acknowledgement

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

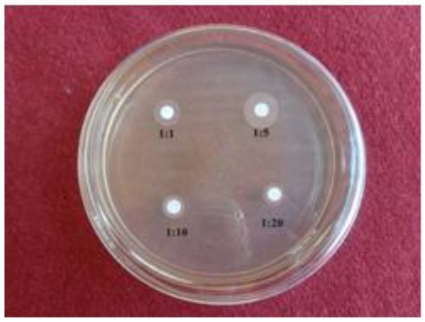
Sl. No.	Essential Oils concentration	Bacillus cereus (gram +ve)	Proteus vulgaris (gram –ve)
1	Clove oil		
	1:1	4	4
	1:5	6	6
	1:10	3	3
	1:20	2	1
2	Olive oil		
	1:1	5	4
	1:5	4	3
	1:10	3	2

	1:20	3	2
3	Orange oil		
	1:1	2	2
	1:5	2	2
	1:10	3	2
	1:20	2	3
4	Rose oil		
	1:1	3	1
	1:5	5	2
	1:10	5	2
	1:20	6	3
5	Lemongrass oil		
	1:1	3	2
	1:5	4	2
	1:10	5	3
	1:20	2	1
6	Heppa oil		
	1:1	2	2
	1:5	2	2
	1:10	3	3
	1:20	4	1
7	Lavender oil		
	1:1	4	2
	1:5	7	3
	1:10	8	4
	1:20	5	2
8	Cinnamon oil		
	1:1	3	2
	1:5	5	4
	1:10	8	6
	1:20	6	5

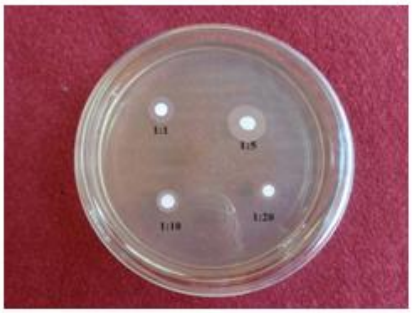
Data are the mean values of four replicates (diameter of inhibition zone (mm)

Antibacterial activity of Essential Oils on Bacillus cereus and Proteus vulgaris

Plate – I

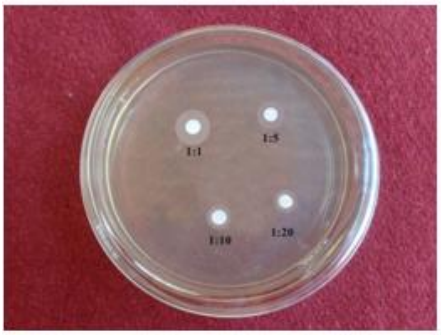


Bacillus cereus (Gram +ve)

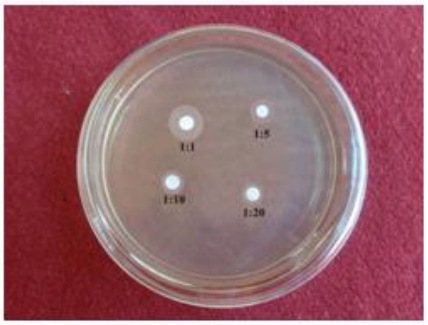


Proteus vulgaris (gram –ve)

Plate –II: **OLIVE OIL**



Bacillus cereus (Gram +ve)



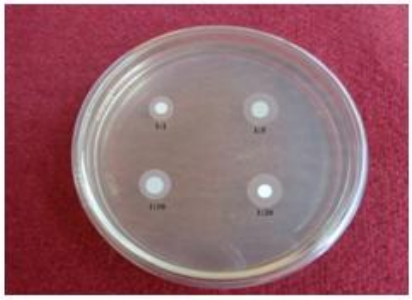
Proteus vulgaris (gram –ve)

Plate –III: **ORANGE OIL**

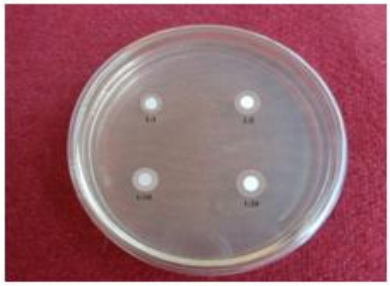
Bacillus cereus (Gram +ve)

Proteus vulgaris (gram –ve)

Plate –IV: **Rose Oil**

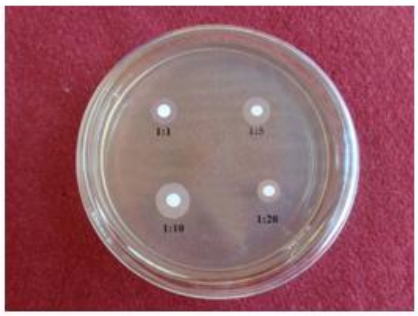


Bacillus cereus (Gram +ve)

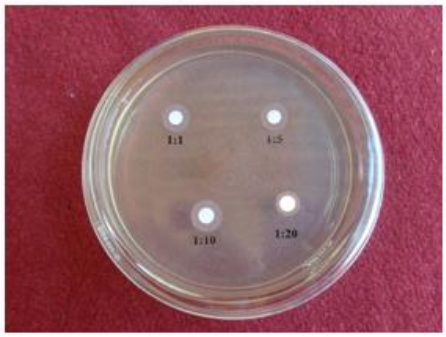


Proteus vulgaris (gram –ve)

Plate –V: **Lemongrass Oil**

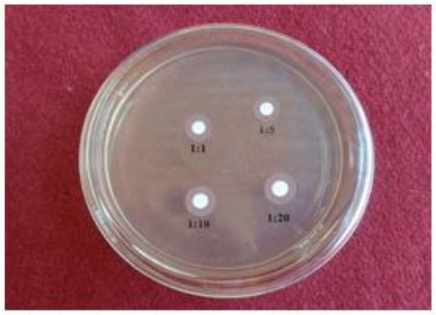


Bacillus cereus (Gram +ve)

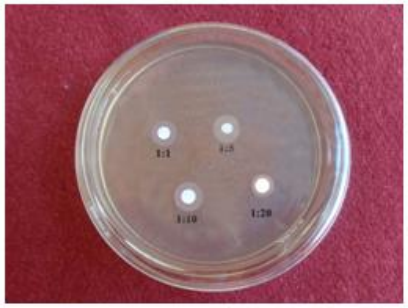


Proteus vulgaris (gram –ve)

Plate –VI: **Heppa Oil**

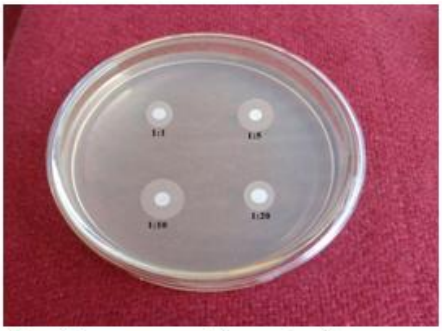


Bacillus cereus(Gram +ve)

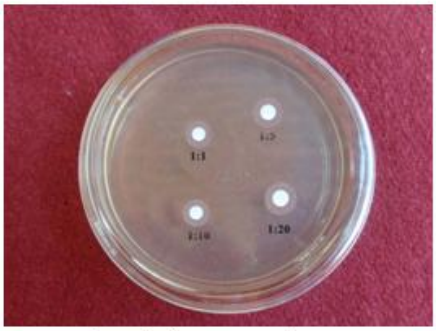


Proteus vulgaris (gram –ve)

Plate –VII: **Lavender Oil**

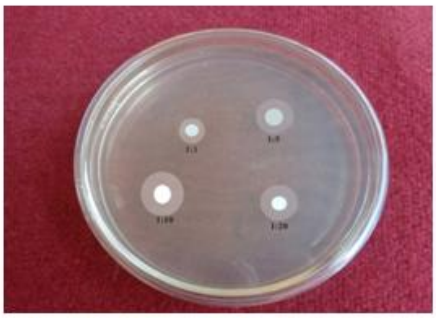


Bacillus cereus (Gram +ve)

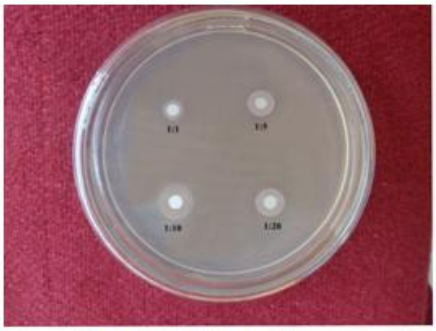


Proteus vulgaris (gram –ve)

Plate –VIII: **Cinnamon Oil**



Bacillus cereus (Gram +ve)



Proteus vulgaris (gram –ve)

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