

**ESSENTIAL OILS AS AN ALTERNATIVE TO
SYNTHETIC PESTICIDE FOR CONTROL OF
PATHOGENIC FUNGI AND NEMATODE**

by

ARCHANA SINHA

Centre for Rural Development and Technology

Submitted

in fulfilment of the requirements of the degree of Doctor of Philosophy

to the

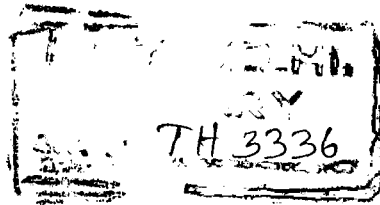


**INDIAN INSTITUTE OF TECHNOLOGY, DELHI
HAUZ KHAS, NEW DELHI
DECEMBER, 2005**

Aromatic plants - Natural pesticides

Biopesticides - Essential oils - Fungicide & Nematicides

Essential oils - pest control agents



TH

665.52 : 595.132

SIN-E

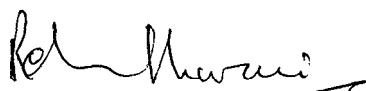


Dedicated
To
My
Beloved Family

CERTIFICATE

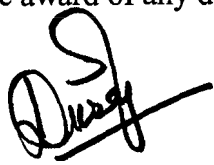
This is to certify that the thesis entitled “**ESSENTIAL OILS AS AN ALTERNATIVE TO SYNTHETIC PESTICIDE FOR CONTROL OF PATHOGENIC FUNGI AND NEMATODE**” being submitted by **Ms. Archana Sinha** to the Indian Institute of Technology, Delhi for the award of Doctor of Philosophy is a record of bonafide research work carried out by her under our guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi.

The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.



(Prof. R. C. Maheshwari)

Supervisor
Centre for Rural Development and Technology
Indian Institute of Technology, Delhi
New Delhi – 110 016.



(Dr. Prem Dureja)

Co-supervisor
Division of Agricultural Chemicals
Indian Agricultural Research Institute
New Delhi – 110 012.

ACKNOWLEDGEMENT

It is by Gods grace and the will of the Almighty that I take this opportunity to express my deep sense of gratitude with reverence to all those who directly or indirectly have helped me throughout the research programme.

My diction is too poor to translate my esteemed and profound sense of gratitude into words for the judicious and precious advice, constructive criticism, constant encouragement, concrete suggestions and painstaking supervision throughout the course of investigation and preparation of manuscript extended by my supervisors Prof. R.C. Maheshwari, Professor, Centre for Rural Development and Technology, Indian Institute of Technology, Delhi and Dr. (Ms.) Prem Dureja, Principal Scientist, Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi.

I express my sincere bouquet of gratitude to Dr. (Mrs.) Vijayalakshmi Mojumder, Principal Scientist, Division of Nematology, IARI, for providing necessary facilities, invaluable guidance and kind help during the bioassay studies and above all for her cordial and motherly treatment which goes much beyond her formal obligations.

I express my sincere thanks to Prof Santosh Satya, Head, CRDI, IIT and other faculty members of CRDI who extended all possible help in my difficult times and encouraged me always throughout the research work.

I thank Mr. S. C. Varshney, Som Shanti Chemicals Ltd, Noida and Dr. S. Walia, Principal Scientist, Division of Agricultural Chemicals, IARI, New Delhi without whose support spectral analysis would not have been possible.

I am greatly grateful to Dr. Badri Prasad and Dr. N. C. Chaudhury (Ex- Directors Central Revenues Control Laboratory, New Delhi) whose blessing and encouragement has always been a

treasure for me. I am equally indebted to my office colleagues Mr. R. K. Adhikari, Mr. M.P. Bansal, Mrs. Meenakshi Gupta and friends Sanjukta and Kanchan for their kind co-operation as a family member.

I shall be failing in my duty if I don't acknowledge the help of Dr. Yadvika for her kind co-operation, encouragement and ready gesture to help throughout the research work,

I thank all the family members of IARI and IIT labs – Dr. Sugatha Roy, Mrs. Shyamli Singh, Dr. Aarti Mahajan, Dr. Kaushal Gautam, Dr. Anshuman Maity, Dr. Anuja Bhardwaj, Dr. Meenakshi Arora, Mrs. Neetu, Rajan and Sh. Karan Singh for their constructive help throughout the course of my study.

I owe a special debt of gratitude to Mr. Vishal Khataavkar for giving me valuable suggestions and wholehearted help at every step of the research programme.

Steady patience, constant encouragement and encompassing love and affection bestowed on me by Ammaji, Pitaji, Bhaiya, Anita, Jiju, Ranjana, Dibu and Titu who are instrumental in bringing me to this pursuit of studies. Their eternal fountain of blessings, never ending sacrifice and constant back up kept me enthusiastic throughout my educational career.

There are many others who have supported and helped me in various ways. I sincerely thank all of them and request their forgiveness for not mentioning them here by name.

Inspite of all this valuable guidance and help, errors must remain which are my domain of responsibility.

Archana Sinha
Archana Sinha

ABSTRACT

Natural pesticides based on essential oils of aromatic plants have been in use since decades for the control of plant diseases and insect pests. These may prove alternative crop protectants to hazardous and non-eco friendly synthetic pesticides which suffer from several drawbacks like pest resistance and resurgence, contamination of food and environment, undesirable effects on the non-target biota and the like. In view of this, the present study on chemical composition and biological activity of essential oils and their constituents was undertaken to evaluate their potential as fungicide and nematocide under laboratory, green house and microplot conditions.

The essential oil of *Acorus calamus* (Sweet Flag) extracted from the rhizomes by Clevenger method showed *cis* and *trans*-asarone as its major constituents when analysed by GC-MS. Commercially available oils used in the study namely lemongrass, jamrosa, basil, Japanese mint, bergamot mint and spearmint essential oils were analysed for their composition and component profile by GC-MS. Citral and geranyl acetate were found to be the major components of lemongrass oil, whereas geraniol, piperitone, geranyl acetate and linalool were the most prevalent components of jamrosa oil and menthol and menthone were the major constituents of Japanese mint oil. Spearmint and bergamot mint, which also belong to the genus *Mentha* contained *l*-carvone, limonene and linalyl acetate, linalool, respectively as their major components. Methyl chavicol and linalool were identified as the major chemical constituents of basil oil.

Essential oils and their pure constituents were evaluated for their antifungal activity against soil borne pathogenic fungi namely, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Rhizoctonia bataticola* and *Macrophomina phaseolina*. Lemongrass oil and *trans*-asarone were found to be most effective against all the test fungi and basil oil, linalool and menthone were least active. All these essential oils and their major components exhibited maximum antifungal activity against *S. rolfsii*, which is a devastating fungi that causes diseases like damping-off of seedlings, stem canker, crown blight, root, crown, bulb and tuber rot, and fruit rots.

All the essential oils and their major components when assayed for nematocidal activity exhibited dose and time dependent activity against the second stage juveniles (J₂) of root knot nematode *Meloidogyne incognita*. Basil oil and *trans*-asarone were found to be the most active and bergamot mint oil and linalool were the least effective after 12, 24 and 48 h of exposure. Effect of the essential oils against J₂ of *M. incognita* was irreversible, as they did not revive after fresh water treatment.

Drenching the soil with different concentrations of essential oils (whole) in plastic cups showed significant reduction in the penetration and development of *M. incognita* in green gram (*Vigna radiata* cv. Pusa Baisakhi). All the test oils except for bergamot mint oil considerably reduced the gall formation, nematode population inside the root, female formation and egg masses formation.

In the *in vitro* studies, maximum antifungal and nematocidal activity was exhibited by lemongrass and basil oils, respectively therefore their microemulsion and granular formulations were prepared to further evaluate their bioefficacy in *in vivo*

studies. Microemulsion formulation of both the oils was prepared by using non-ionic surfactants (Triton X-100 + Tween 80) and co-surfactant (butanol). Whereas, sodium alginate based granular formulation was prepared by interfacial insolubilization reaction using kaolin as inert ingredient. Microemulsion formulation qualified the required physico-chemical properties such as emulsion stability, flash point and acidity / alkalinity as prescribed by Bureau of Indian Standards (BIS). The microemulsion formulation of lemongrass oil when stored at room temperature showed half-life of 188.1 days. Release of lemongrass oil in water from alginate based granular formulation (on the basis of citral content) showed a half-life of 9.21 days.

Microemulsion and granular formulations of lemongrass oil effectively inhibited the growth of *S. rolfii* in laboratory studies and hence their efficacy was evaluated *in vivo*. Both the formulations significantly prevented tomato damping-off in pot culture studies. Plant stand with microemulsion formulation treatment was at par with the non- infested control and was superior over granular formulation. Moreover, plants treated with microemulsion formulation were found to be healthier as compared to the granular formulation.

The bioefficacy of basil and lemongrass oils based microemulsion and granular formulations was also assessed against *M. incognita* in tomato plants. Microemulsion formulation of both the oils was more effective in controlling nematode infection as compared to the granular formulation.

The microemulsion formulation of basil and lemongrass oils were further tested under microplot conditions by drenching the soil with formulations 10 days before

sowing green gram (*Vigna radiata* cv. Pusa Baisakhi) seeds. The formulations significantly reduced the gall formation as compared to untreated microplot plants. Maximum root gall reduction was observed with basil oil. Significant increase in the number of bacterial nodules, plant height and yield was also observed with basil oil. The population of *M. incognita* in soil was considerably reduced with all the treatments. An increase in the population of non-parasitic nematodes was also recorded.

In view of their significant antifungal and nematicidal activity, essential oils hold good promise for possible use as pest control agents.

CONTENTS

	Page No.
Acknowledgement	i
Abstract	iii
List of Figures	vii
List of Plates	ix
List of Tables	x
Notations	xiii
1. Introduction	1
2. Literature Review	5
2.1 Essential oils	5
2.1.1 Extraction of essential oils	8
2.1.1.1 Conventional techniques	8
2.1.1.2 New techniques	9
2.1.2 Chemical composition of essential oils	11
2.1.2.1 <i>Acorus</i> L. (Araceae)	13
2.1.2.2 <i>Cymbopogon</i> sp. (Poaceae)	14
2.1.2.3 <i>Mentha</i> L. (Lamiaceae)	16
2.1.2.4 <i>Ocimum</i> L. (Lamiaceae)	18
2.1.2.5 Terpenes and Terpenoids	20
2.2 Problem of soil borne pathogens: Fungi and Nematodes	25
2.3 Biological activity of essential oils and their constituents	29
2.3.1 Fungicidal activity	30
2.3.2 Nematicidal activity	36
2.3.3 Insecticidal activity	39
2.3.4 Antibacterial activity	42
2.3.5 Antiviral activity	44
2.4 Structure- activity relationship (SAR) studies	44
2.5 Formulations	46
2.5.1 Microemulsions	47
2.5.2 Controlled Release Formulations	48
2.5.3 Commercial exploitation of essential oil	49
2.6 Relevance of the present investigations	52

3. Materials and Methods	53
3.1.1 Extraction of essential oil of <i>Acorus calamus</i> rhizome	54
3.1.2 Characterization of oils by GC-MS	55
3.3 Bioactivity Evaluation	55
3.3.1 Antifungal activity	55
3.3.2 Nematicidal activity	60
3.3.3 Preliminary trial on the effect of oils on viability of J ₂ in soil and their development in susceptible host (Green gram)	62
3.4 Preparation of formulations	65
3.4.1 Microemulsion formulations	65
3.4.2 Physico chemical properties of microemulsion formulation	66
3.4.3 Granular formulation	69
3.4.4 Release of lemongrass oil from the alginate matrix	70
3.5 Bioefficacy tests of essential oil formulations	71
3.5.1 Antifungal activity of lemongrass oil based formulations	71
3.5.2 Efficacy of lemongrass and basil oil based formulations against <i>M. incognita</i>	74
4. Results and Discussion	78
4.1 Hydrodistillation of <i>Acorus calamus</i> rhizomes	78
4.2 Characterization of essential oils by GC-MS	78
4.2.1 Sweet flag oil	79
4.2.2 Lemongrass oil	81
4.2.3 Jamrosa oil	90
4.2.4 Japanese mint oil	97
4.2.5 Spearmint oil	104
4.2.6 Bergomot mint oil	111
4.2.7 Basil oil	119
4.3 Biological activity	125
4.3.1 Antifungal activity	125
4.3.2 Nematicidal activity	151
4.4 Formulations	173
4.4.1 Microemulsion formulation	173

4.4.2 Granular formulation	177
4.4.3 Bio efficacy tests of formulations	180
5. Summary and Conclusions	194
Bibliography	206
Biodata	