

ANTIFUNGAL EFFECT OF LEMONGRASS (*Cymbopogon citratus*) ESSENTIAL OIL ON SEED ASSOCIATED FUNGI OF WHEAT AND RICE



By

MOHAMMAD ISHAQ

**Department of Earth and Environmental Sciences Bahria University,
Islamabad**

2013

Dedicated to my beloved parents and my loving wife Mrs. Shehla, Gul who really supported me throughout my MS research work.

ABSTRACT

Rice and wheat germplasm were screened for the presence of seed both saprophytic as well as pathogenic fungi associated with wheat and rice seeds. Seven fungal species viz., *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Drechslera indica*, *Drechslera tripogonis* and *Fusarium moniliforme* were associated with rice while five fungi viz., *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Drechslera indica* were found in wheat. Among all the isolated fungi *Aspergillus flavus* was found as predominant fungi (41%) associated with seeds of rice and (33%) with seeds of wheat. The antifungal potential at different concentrations of lemongrass essential oil was tested by using disk diffusion method. Minimum inhibitory concentration (MIC) of lemongrass essential oil against each fungus was determined through agar plug method. Highest MIC (55ppm) was recorded for *Aspergillus flavus* and lowest for *Drechslera indica* (20ppm). Significant differences were recorded for different concentrations of lemongrass essential oil against isolated fungi ($p \leq 0.05$) against tested fungi. However, *Aspergillus niger* was found the most susceptible fungal strain to lemongrass essential oil.

ACKNOWLEDGEMENTS

I am very grateful to my supervisors Mr. Asif Javed, Assistant Professor, Department of Earth and Environmental Sciences (E&ES), Bahria University, Islamabad and Mrs. Riffat Tahira, Senior Scientific Officer, Plant Genetics Resources Institute (PGRI), National Agriculture Research Centre (NARC) Islamabad for their guidance and useful suggestions throughout my research work. I will always remember the generous attitude of Mr. Faisal Sohail Fateh, Senior Scientific Officer, IPEP, NARC, who extended full cooperation for the identification of fungi.

I am also thankful to Dr. Muhammad Zafar, Head of Department (E&ES) for facilitating my research work. I extend my gratitude to Prof. Dr. Tahseenullah Khan (E&ES) who reviewed my thesis critically and offered fruitful suggestions for the improvement.

I would also like to thank Mr. Izhar Muhammad and Miss. Tauseef Rehan for their help and support during my lab work. I pay cordial thanks to my brother Mr. Muhammad Saeed Lecturer at GDC Khair-abad (Mardan) for his moral support.

ABBREVIATIONS

µm	microliter
µm	micrometer
ml	milliliter
mm	millimeter
° C	degree Celsius
cm	centimeter
DMSO	dimethyl sulphoxide
KPK	Khyber Pukhtunkhwa
NARC	National Agriculture Research Center
PDA	potato dextrose Agar
PGRI	Plant Genetic Resources Institute
MIC	minimum inhibitory concentration
ppm	part per million
PC	positive control

CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	ii
ABBREVIATIONS	iii
CONTENTS	iv
FIGURES	vii
TABLES	viii

CHAPTER 1

INTRODUCTION

1.1	Background	1
1.2	Fungi associated with wheat seeds	1
1.3	Fungi associated with rice seeds	2
1.4	Antimicrobial studies for essential oils and plant extract	4
1.5	Objectives of the study	5

CHAPTER 2

MATERIALS AND METHODS

2.1	Acquisition of seed samples	7
2.2	Isolation of seed associated fungi	7
2.3	Purification of cultures	7

2.4	Identification of fungi	8
2.5	Collection of plant material for essential oil extraction	8
2.6	Essential oil extraction	8
2.7	Preparation of stock solution of essential oil and antibiotic	8
2.8.1	Determination of minimum inhibitory concentration (MIC) of essential oil	9
2.8.2	Antifungal potential through disk diffusion method	9
2.9	Statistical analysis	9

CHAPTER 3

RESULTS AND DISCUSSION

3.1	Isolation of fungi	12
3.1.1	Isolation of fungi from wheat seeds	12
3.1.2	Isolation of fungi from rice seeds	21
3.2	Identification of fungi	29
3.2.1	<i>Alternaria alternata</i> (Fr.Keissel)	29
3.2.2	<i>Aspergillus flavus</i> (Link ex Gray)	29
3.2.3	<i>Aspergillus fumigatus</i> (Link)	30
3.2.4	<i>Aspergillus niger</i> (van Tieghem)	30
3.2.5	<i>Curvularia lunata</i> (Boedijn)	31
3.2.6	<i>Fusarium moniliforme</i> (Sacc)	31

3.2.7	<i>Drechslera indica</i> (Shoemaker)	32
3.2.8	<i>Drechslera tripogonis</i> (A. S. Patil and V. G. Rao)	32
3.3	Antimicrobial assay	33

CHAPTER 4

SUMMARY AND CONCLUSIONS

4.1	Summary	40
4.2	Conclusions	41
	REFERENCES	42

FIGURES

	Page
Figure 3.1. Blotter's test for isolation of seed associated fungi in Wheat (A) and Rice (B).	14
Figure 3.2. Frequency of occurrence of seed associated fungi isolated from wheat.	20
Figure 3.3. Frequency of occurrence of seed associated fungi isolated from rice.	28
Figure 3.4. Morphological characteristics of <i>A. alternata</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	29
Figure 3.5. Morphological characteristics of <i>A. flavus</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	30
Figure 3.6. Morphological characteristics of <i>A. fumigatus</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	30
Figure 3.7. Morphological characteristics of <i>A. niger</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	31
Figure 3.8. Morphological characteristics of <i>C. lunata</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	31
Figure 3.9. Morphological characteristics of <i>F. moniliforme</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	32
Figure 3.10. Morphological characteristics of <i>D. indica</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	32
Figure 3.11. Morphological characteristics of <i>D. tripogonis</i> (A) Pure culture. (B) Photomicrograph showing conidia at 40X magnification.	33
Figure 3.12. Effect of lemongrass essential oil on mycelial growth of <i>A. flavus</i> .	39
Figure 3.13. Effect of lemongrass essential oil on mycelial growth of <i>D. indica</i> .	39

TABLES

		Page
Table 2.1.	List of wheat accessions used in the study.	10
Table 2.2.	List of rice accessions used in the study.	11
Table 3.1.	Frequency of occurrence of seed associated fungi isolated from wheat.	15
Table 3.2.	Frequency of occurrence of seed associated fungi isolated from rice.	23
Table 3.3.	MIC of lemongrass essential oil for isolated fungal strains through agar method.	34
Table 3.4.	Analysis of variance for antifungal activity of lemongrass essential oil.	34
Table 3.5.	Effect of different concentrations of lemongrass essential oil and antibiotic.	35
Table 3.6.	Inhibitory effect of lemongrass essential oil and fluconazole on fungal strains.	36
Table 3.7.	Mean values for zone of inhibition produced by different concentrations of lemongrass essential oil/antibiotic against isolated fungal strains.	38