

TABLE OF CONTENTS

GENERAL INTRODUCTION	1
1 Banana and its importance	1
1.1 Botanical description	2
2 Nematode pests of banana	3
2.1 Burrowing nematode <i>Radopholus similis</i>	3
2.2 Threshold and control measures for nematodes in banana	4
3 Biological approaches for sustainable nematode management in banana.....	5
3.1 Mutualistic fungal endophytes	6
3.2 Opportunistic egg-pathogenic fungi.....	6
3.3 Antagonistic bacteria.....	7
3.3.1 Rhizobacteria.....	8
3.3.2 Endophytic bacteria.....	8
3.3.3 Opportunistic parasitic bacteria.....	9
4 Scope of the study	11
5 References.....	12
GENERAL MATERIALS AND METHODS	22
1 Antagonistic biocontrol agents	22
1.1 Origin and culture of <i>Bacillus firmus</i>	22
1.1.1 Bacteria culture and identification	22
1.2 Origin and culture of <i>Fusarium oxysporum</i> strain 162	22
1.3 Origin and culture of <i>Paecilomyces lilacinus</i> strain 251.....	23
2 Nematodes	23
2.1 Origin and culture of <i>Radopholus similis</i>	23
2.1.1 Preparation of the inoculum	24
2.2 Origin and culture of <i>Meloidogyne incognita</i>	24
2.2.1 Preparation of the inoculum	25
2.3 Origin and culture of <i>Ditylenchus dipsaci</i>	25
2.3.1 Preparation of the inoculum	25
3 Plants used for experiments	26
3.1 Banana plantlets	26
3.2 Tomato plants.....	27
4 Culture media and reagents.....	28
5 Statistical analysis.....	29
6 References.....	30
IN VITRO ACTIVITY OF BACILLUS FIRMUS AGAINST RADOPHOLUS SIMILIS, MELOIDOGYNE INCOGNITA, DITYLENCHUS DIPSACI AND THEIR POSSIBLE MODES-OF-ACTION	31
1 Introduction.....	31
2 Materials and Methods.....	33
2.1 Effect of cell-free water extracts of two <i>Bacillus firmus</i> formulations on nematode paralysis and mortality.....	33
2.2 Effect of cell-free culture filtrates from <i>Bacillus firmus</i> fermentation on nematode paralysis and mortality	33
2.3 Effect of cell-free culture filtrates from <i>Bacillus firmus</i> fermentation on hatching viability of <i>Meloidogyne incognita</i> eggs.....	34

2.4	Effect of <i>Bacillus firmus</i> cell suspensions on <i>Radopholus similis</i> mortality in sand	35
2.5	<i>Bacillus firmus</i> protease and chitinase activity	35
2.5.1	Preparation of chitin suspension	36
2.6	Soil extract and organic matter-water extract media.....	36
2.7	Culture media and reagents	37
3	Results.....	38
3.1	Effect of cell-free water extracts of two <i>Bacillus firmus</i> formulations on nematode paralysis and mortality.....	38
3.2	Effect of cell-free culture filtrates from <i>Bacillus firmus</i> fermentation on nematode paralysis and mortality	40
3.3	Effect of cell-free culture filtrates from <i>B. firmus</i> fermentation on hatching viability of <i>Meloidogyne incognita</i> eggs.....	42
3.4	Effect of <i>Bacillus firmus</i> cell suspensions on <i>Radopholus similis</i> mortality in sand.....	43
3.5	<i>Bacillus firmus</i> protease and chitinase activity	44
4	Discussion.....	48
4.1	Effect of cell-free water extracts of two <i>Bacillus firmus</i> formulations on nematode paralysis and mortality.....	48
4.2	Effect of cell-free culture filtrates from <i>Bacillus firmus</i> fermentation on nematode paralysis and mortality	48
4.3	Effect of cell-free culture filtrates from <i>Bacillus firmus</i> fermentation on hatching viability of <i>Meloidogyne incognita</i> eggs.....	49
4.4	Effect of <i>Bacillus firmus</i> cell suspensions on <i>Radopholus similis</i> mortality in sand.....	49
4.5	<i>Bacillus firmus</i> protease and chitinase activity	50
5	Conclusions.....	51
6	References.....	52

EFFECT OF TREATMENT METHODOLOGIES ON THE COLONIZATION OF *FUSARIUM OXYSPORUM* STRAIN 162 ON BANANA..... 56

1	Introduction.....	56
2	Materials and Methods.....	58
2.1	Substrates	58
2.2	<i>Fusarium oxysporum</i> re-isolation.....	58
2.3	<i>In vitro</i> compatibility between <i>Fusarium oxysporum</i> , <i>Paecilomyces lilacinus</i> and <i>Bacillus firmus</i>	59
2.4	<i>Fusarium oxysporum</i> colonization dynamics over time	59
2.5	Effect of inoculum density on <i>Fusarium oxysporum</i> root colonization.....	60
2.6	Effect of the application form on <i>Fusarium oxysporum</i> colonization and distribution in the roots	60
2.7	Effect of <i>Paecilomyces lilacinus</i> or <i>Bacillus firmus</i> on <i>Fusarium oxysporum</i> colonization	61
3	Results.....	63
3.1	<i>In vitro</i> compatibility of <i>Fusarium oxysporum</i> , <i>Paecilomyces lilacinus</i> and <i>Bacillus firmus</i>	63
3.2	<i>Fusarium oxysporum</i> colonization over time.....	64
3.3	Effect of inoculum density on <i>Fusarium oxysporum</i> root colonization.....	65
3.4	Effect of the application form on <i>F. oxysporum</i> distribution in the roots and colonization	65

3.5	Effect of <i>Paecilomyces lilacinus</i> or <i>Bacillus firmus</i> on <i>Fusarium oxysporum</i> colonization	68
4	Discussion.....	70
4.1	<i>In vitro</i> compatibility between <i>Fusarium oxysporum</i> , <i>Paecilomyces lilacinus</i> and <i>Bacillus firmus</i>	70
4.2	<i>Fusarium oxysporum</i> colonization over time.....	70
4.3	Effect of inoculum density on <i>Fusarium oxysporum</i> root colonization.....	71
4.4	Effect of the application form on <i>Fusarium oxysporum</i> distribution in the roots and colonization	71
4.5	Effect of <i>Paecilomyces lilacinus</i> or <i>Bacillus firmus</i> on <i>Fusarium oxysporum</i> colonization	72
5	Conclusions.....	73
6	References.....	74

BIOLOGICAL CONTROL OF THE BURROWING NEMATODE *RADOPHOLUS SIMILIS* IN BANANA BY SINGLE OR COMBINED APPLICATIONS OF THE ANTAGONISTS *FUSARIUM OXYSPORUM* STRAIN 162, *PAECILOMYCES LILACINUS* STRAIN 251 AND *BACILLUS FIRMUS*..... 77

1	Introduction.....	77
2	Materials and Methods.....	79
2.1	Microorganisms.....	79
2.2	Substrate	79
2.3	Extraction of nematodes from root samples and the pot soil	79
2.4	Dose-response of <i>Radopholus similis</i> to <i>Bacillus firmus</i>	80
2.5	Effect of sequential applications of <i>Paecilomyces lilacinus</i> on <i>Radopholus similis</i> penetration.....	81
2.6	Effect of sequential applications of <i>Paecilomyces lilacinus</i> on the second generation of <i>Radopholus similis</i>	81
2.7	Effect of dual-applications of <i>Fusarium oxysporum</i> , <i>Paecilomyces lilacinus</i> or <i>Bacillus firmus</i> on biocontrol efficacy	82
2.7.1	<i>Fusarium oxysporum</i> and <i>Paecilomyces lilacinus</i>	82
2.7.2	<i>Fusarium oxysporum</i> and <i>Bacillus firmus</i>	83
2.8	Simultaneous applications of <i>Paecilomyces lilacinus</i> and <i>Bacillus firmus</i>	83
2.8.1	At-planting application of biocontrol agents.....	84
2.8.2	Post-planting application of biocontrol agents.....	84
3	Results.....	85
3.1	Dose-response of <i>Radopholus similis</i> to <i>Bacillus firmus</i>	85
3.2	Effect of sequential applications of <i>Paecilomyces lilacinus</i> on <i>Radopholus similis</i> penetration.....	86
3.3	Effect of sequential applications of <i>Paecilomyces lilacinus</i> on the second generation of <i>Radopholus similis</i>	88
3.4	Effect of dual-applications of <i>Fusarium oxysporum</i> , <i>Paecilomyces lilacinus</i> or <i>Bacillus firmus</i> on biocontrol efficacy	90
3.4.1	<i>Fusarium oxysporum</i> and <i>Paecilomyces lilacinus</i>	90
3.4.2	<i>Fusarium oxysporum</i> and <i>Bacillus firmus</i>	91
3.5	Simultaneous applications of <i>Paecilomyces lilacinus</i> and <i>Bacillus firmus</i>	94
3.5.1	At-planting application of biocontrol agents.....	94
3.5.2	Post-planting application of biocontrol agents.....	97
4	Discussion.....	100
4.1	Dose-response of <i>Radopholus similis</i> to <i>Bacillus firmus</i>	100

4.2	Effect of sequential applications of <i>Paecilomyces lilacinus</i> on <i>Radopholus similis</i> penetration and reproduction.....	100
4.3	Effect of dual-applications of <i>Fusarium oxysporum</i> , <i>Paecilomyces lilacinus</i> or <i>Bacillus firmus</i> on biocontrol efficacy.....	101
4.3.1	<i>Fusarium oxysporum</i> and <i>Paecilomyces lilacinus</i>	101
4.3.2	<i>Fusarium oxysporum</i> and <i>Bacillus firmus</i>	102
4.4	Simultaneous applications of <i>Paecilomyces lilacinus</i> and <i>Bacillus firmus</i>	102
4.4.1	At-planting application of biocontrol agents.....	102
4.4.2	Post-planting application of biocontrol agents.....	103
5	Conclusion	105
6	References.....	106

EFFECT OF SUBSTRATE TYPE AND THE PRESENCE OF THE ANTAGONISTIC BACTERIA *BACILLUS FIRMUS* ON THE PERSISTENCE OF *PAECILOMYCES LILACINUS* STRAIN 251 OVER TIME 111

1	Introduction.....	111
2	Materials and Methods.....	113
2.1	Re-isolation of <i>Paecilomyces lilacinus</i>	113
2.2	Effect of sand amended with organic matter on the persistence of <i>Paecilomyces lilacinus</i>	113
2.3	Effect of <i>Bacillus firmus</i> on the persistence of <i>Paecilomyces lilacinus</i> in sand amended with organic matter	114
2.4	Effect of clay soil amended with organic matter on the persistence of <i>Paecilomyces lilacinus</i>	114
2.5	Effect of <i>Bacillus firmus</i> on the persistence of <i>Paecilomyces lilacinus</i> in clay soil amended with organic matter	115
2.6	Statistical analysis	115
3	Results.....	117
3.1	Effect of sand amended with organic matter and the presence of <i>Bacillus firmus</i> on the persistence of <i>Paecilomyces lilacinus</i>	117
3.2	Effect of clay soil amended with organic matter and the presence of <i>Bacillus firmus</i> on the persistence of <i>Paecilomyces lilacinus</i>	119
4	Discussion.....	121
4.1	Effect of organic matter content and the presence of <i>Bacillus firmus</i> on the persistence of <i>Paecilomyces lilacinus</i> in sand or clay soil.....	121
5	Conclusion	122
6	References.....	123