

Contents

	Page
1-INTRODUCTION.....	1
2-REVIEW.....	3
3-MATERIALS AND METHODS	
3.1. Isolation of microorganisms.	
3.1.1. Soil samples.....	18
3.1.2. Treatment of soil samples.....	18
3.1.3. Isolation of actinomycetes from natural habitat	18
3.2.Isolation from faba bean and pathogenicity test.	20
3.2.1. Isolation of pathogenic fungi from faba bean leaves	20
3.2.2. Identification of the causal agent of chocolate spot disease on faba bean	21
3.2.3. Pathogenicity test of <i>Botrytis</i> sp.....	21
3.3.The anti-microbial potentialities of actinomycetes isolates	23
3.3.1. The anti-microbial activities of the used actinomycetes isolates.....	23
3.3.1.1. Media used for investigating anti-microbial activities.....	23
3.3.1.2. Estimation of antifungal producing actinomycetes.....	25
3.3.2. Taxonomical and biological studies of the selective actinomycete isolate.....	26
3.3.2.1. Identification of genera.....	26
3.3.2.1.1. Methods used for morphological studies.....	26
3.3.2.1.2. Analysis of cell wall hydrolysate the detection of Diaminopimelic Acid and sugars.....	27
3.3.2.2. The methods used for the classification of genus <i>Streptomyces</i>	28
3.3.2.2.1. Macroscopic characteristics and pigmentation of culture media...	28
3.3.2.2.2. Culture characteristics.....	29
3.3.2.2.3. Microscopic characteristics.....	30

3.3.2.2.4. <i>Physiological and bio-chemical properties</i>	30
3.4. Production, extraction, purification and characterization of the anti-microbial product produced by the selective isolate of actinomycete .	37
3.4.1. <i>Effect of certain factors on the anti-microbial bio-activity produced by selective isolate of actinomycete</i>	37
3.4.1.1. <i>The effect of different incubation periods</i>	37
3.4.1.2. <i>The effect of different hydrogen ion concentrations</i>	38
3.4.1.3. <i>The effect of different incubation temperatures</i>	38
3.4.2. <i>The production of the anti-microbial material</i>	38
3.4.2.1. <i>Effect of different media on production of the anti-microbial product on shaken cultures</i>	38
3.4.2.2. <i>Production of the anti-microbial product in fermentor</i>	39
3.4.3. Extraction and purification of the anti-microbial product produced by selective actinomycete isolate	40
3.4.3.1. <i>Extraction of the anti-microbial product</i>	40
3.4.3.2. <i>Purification of the anti-microbial product</i>	40
3.4.4. The bio-autography of the purified anti-microbial product produced by selective actinomycete isolate	40
3.4.5. The antibiotic potentialities of the anti-microbial product produced by the selective actinomycete isolate	41
3.4.5.1. <i>Determination of minimum inhibitory concentration and minimum bactericidal concentration of the anti-microbial product</i>	41
3.4.5.2. <i>Determination of the anti-microbial activities of the product produced by the selective isolate of actinomycete on chocolate spot disease</i>	42
3.5.Characterization of the anti-microbial product produced by the selective isolate of actinomycete	43
3.5.1. <i>Physicochemical characteristics of the purified anti-microbial product</i>	43

3.5.2. The spectroscopic analysis of the purified anti-microbial product...	44
3.5.3. The chemical analysis of the purified anti-microbial product... ..	44
3.5.3.1. Detection of amino acids content... ..	45
3.5.3.2. Detection of sugar content	45
3.5.3.3. Determination of proteins... ..	45
3.5.4. The behaviour of anti-microbial products towards certain chemical tests.....	46
Index of experimental media and reagents	49
4- RESULTS	
4.1. Isolation of soil microorganisms	61
• Isolation of microorganisms tolerance to fungicide	61
• Effect of different concentrations of fungicide on the growth of isolated actinomycetes	65
4.2. Isolation from faba bean plants and pathogenicity test of <i>Botrytis</i> isolates	67
4.2.1. Isolation of the causal organism of chocolate spot disease.....	67
4.2.2. Pathogenicity test of <i>Botrytis</i> isolate.....	67
4.3. Anti-microbial potentialities of actinomycetes isolated from soil.	70
4.3.1. Survey of anti-microbial activities.....	70
4.3.2. Antagonistic activities of six actinomycetes isolates against <i>Botrytis fabae</i>	73
4.3.3. Bio-activities of six actinomycetes isolates for controlling chocolate spot disease	77
4.4. Taxonomical and biological studies of the most potent actinomycete isolate(T₁₁₈) producing anti-microbial metabolites.....	81
4.4.1.The detection of diaminopimelic acid and sugar in cell wall hydrolysate of isolate T ₁₁₈	81
4.4.2. Morphological characteristics of the isolate T ₁₁₈	81
4.4.3. Cultural properties.....	83
4.4.4. Physiological and biochemical characteristics of the isolate T ₁₁₈	83

4.5. Production, extraction, purification and characterization of the anti-microbial product produced by <i>S.violaceus</i> T₁₁₈.....	93
4.5.1. Effect of certain factors on the anti-microbial product produced by <i>S.violaceus</i> T ₁₁₈	93
4.5.1.1. Effect of different incubation periods on growth rate and the anti-microbial activities of <i>S.violaceus</i> T ₁₁₈ against <i>Botrytis fabae</i> ...	93
4.5.1.2. Effect of different hydrogen ion concentrations (pH) on growth rate the anti- microbial activities of <i>S.violaceus</i> T ₁₁₈ against <i>Botrytis fabae</i>	95
4.5.1.3. Effect of different temperature on biosynthesis of <i>S.violaceus</i> D ₁₁₈ and bioactivities product against <i>Botrytis fabae</i>	97
4.5.1.4. production of the anti-microbial product in shaken cultures and fermentort	99
• Effect of different media on the bio-activity of the anti-microbial substance produced by <i>S.violaceus</i> T ₁₁₈	99
4.5.2. Extraction and purification of the anti-microbial product	102
4.5.2.1. The Bioautography of the obtained anti-microbial product	102
4.5.2.2. The anti-microbial potentialities of the product produced by <i>S.violaceus</i> T ₁₁₈	102
4.5.3. Control of chocolate spot disease by using the anti-microbial product produced by <i>S.violaceus</i> T ₁₁₈	106
4.6.Characterization of the anti-microbial product produced by <i>S.violaceus</i> T₁₁₈	112
4.6.1.The physiochemical characteristics of the purified anti-microbial product produced by <i>S.violaceus</i> T ₁₁₈	112
4.6.1.1 The physical properties.....	112
4.6.1.2 The spectroscopic analysis of the purified anti-microbial product.....	112
4.6.2. The chemical analysis of the product	113
4.6.2.1. Detection of amino acids, sugar content and proteins.	113

4.6.3. The behaviour of the anti-microbial product towards certain chemical tests and the nomenclature of the product.....	113
5. DISCUSSION.....	120
6. SUMMARY.....	134
7. ABBREVIATION	138
8. REFERENCES.....	139
9. ARABIC SUMMARY.....	v