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Insecticidal effects of some *Streptomycetes* strains isolated from soil samples against the larvae and adults of the *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera, Chrysomelidae)

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ABSTRACT

Thirty-seven *Streptomycetes* strains isolated from different soils and geographical areas in Turkey were used in this study to investigate the insecticidal effects of their three different solutions (A, B, C) on 1-4 th instar larvae and adults of the *Leptinotarsa decemlineata* (Say.) (Coleoptera: Chrysomelidae). The solutions (A, B, C) of 37 strains were given to larvae (1-4 instar) and adults of *L. decemlineata* through the food plant (potato leaves). Following eight days of bioassay, larvae showed different signs before death; generally the larvae displayed sluggishness, loss of appetite and depth of color. As in larvae, during bioassays, adults showed different signs before death; generally the adults displayed sluggishness, loss of appetite and depth of color.

According to this study; considerable lethal effect of some *Streptomycetes* sp. were observed on the larvae (1-4 instar) of *L. decemlineata,* 93.1% larval mortalities caused by (C) solution of *Streptomycetes* strains M1483 and M1282; 96.6% larval mortalities caused by (B) solution of *Streptomycetes* strain M3024 and 98.3% larval mortalities caused by (A) solution of *Streptomycetes* strain M4010. On the other side, no significant mortality effect of *Streptomycetes* strains was observed on adults of *L. decemlineata*. Only *Streptomycetes* strains (B) solution showed 56.4% mortality at M7002. Results of this study indicate that some *Streptomycetes* strain solutions have a potential use in the bio-control of *L. decemlineata.*

Keywords: *Leptinotarsa decemlineata*, *Streptomycetes* strains, Biological control, Coleoptera

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Topraktan izole edilen bazı *Streptomycetes* türlerinin *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera, Chrysomelidae) ergin ve larvalarına karşı insektisidal etkileri

ÖZ

Bu çalışmada Türkiye'nin farklı toprak ve coğrafik alanlarından izole edilen otuz yedi *Streptomycetes* suşunun üç farklı solüsyonu (A, B, C), *Leptinotarsa decemlineata*'nın (Say.) (Coleoptera: Chrysomelidae) 1-4 instar larva ve erginlerine karşı böcek öldürücü etkilerinin incelenmesi için kullanılmıştır. Otuz yedi suşun solüsyonları (A, B, C), *L decemlineata*'nın larva (1-4 instar) ve erginlerine yiyecek (patates yaprağı) vasıtasıyla verilmiştir. Sekiz günü takip eden bioassay çalışmasında larvalar ölmeden önce farklı belirtiler göstermiş; genellikle hareketlerde yavaşlama sergilemiş, iştah ve renk kaybetmişlerdir. Bioassay çalışması boyunca erginlerde de ölmeden önce larvalar gibi hareketlerde yavaşlama, iştah ve renk kaybı gibi belirtiler gözlenmiştir.

Bu çalışmaya göre; *L. decemlineata* larvalarında *Streptomycetes* suşlarının önemli derecede öldürücü etkisinin olduğu gözlemlenmiştir. Larvalarda, *Streptomycetes* suşlarından M1483 ve M1282'nin (C) solüsyonu %93.1; *Streptomycetes* suşlarından M3024'ün (B) solüsyonu %96.6 ve *Streptomycetes* suşlarından M4010'nun (A) solüsyonu %98.3 ölüm oranı göstermiştir. Öte yandan *L. decemlineata* erginlerinde *Streptomycetes* suşlarının önemli derecede öldürücü etkisinin olmadığı gözlemlenmiştir. Sadece *Streptomycetes* suşlarından (B) solüsyonu M7002'de %56.4 ölüm oranı göstermiştir. Bu çalışmanın sonuçları bazı *Streptomycetes* suşlarının *L. decemlineata'*nın biyokontrolünde kullanılma potansiyeli olduğunu göstermiştir.

Anahtar kelimeler: *Leptinotarsa decemlineata*, *Streptomycetes* suşları, Biyolojik mücadele, Coleoptera

INTRODUCTION

Chemical pesticides have traditionally been used to control pests, but these pesticides have a detrimental effect on the environment. As the use of chemical pesticide is a social issue, the objectives of nutrition, health and environmental quality can be addressed more efficiently by the implementation of integrated pest management techniques (IPM) rather than through current crop protection practices (Norgard 1976). Pesticides play an important role in the stabilization and increase of agricultural yield, but are accused of being a possible source of atmospheric pollution, with residual toxicity to mammals and wildlife. Microbial products with antimicrobial activity are now being applied in every sphere of pesticide use. Thus, some antifungal, antibacterial, insecticidal and herbicidal products used in crop protection have been obtained from microorganisms (Yamaguchi 1992). However, certain insect control problems, such as resistance, toxicity, persistence, which require new and safer pesticides, have led to the development of natural products and their semi synthetic derivatives. *Streptomyces* strains are recognized sources of insecticidal natural products. Other microbial products have been studied specifically for their insecticidal activities, e.g. nikkomycins (Dahn et al. 1976),

prasinons (Box et al. 1973), milbemycins (Takiguchi et al. 1980), and a few of them have been commercialized as anti-parasitic compounds; avermectins (Burg et al. 1979), tetranactin (Ando et al. 1971), valinomycin (Heisey 1988), pyrrolizine derivatives (Jizba et al. 1992), respirantin (Urushibata et al. 1993), piercidin (Takahashi et al. 1968), griseulin (Nair et al. 1993), martinomycin (Carter et al. 1994).

Chitinase is originally an enzyme used by insects to degrade the structural polysaccharide "chitin" during the molting process (Zhang et al. 2002). The largest chitinase activity among bacteria has been observed in species of *Streptomyces*, *Serratia*, *Vibrio*, and *Bacillus* (Reguera and Leschine 2001). Chitinase enzyme is very important in the biological control of insects (Reguera and Leschine 2001) and plant pathogenic fungi (El-Tarabily et al. 2000, El-Tarabily 2003). On the other hand *Streptomycetes* metabolites not only effective against insect but may also protect the insect themselves from other microbial pathogens and insects as in beewolf wasps which cultures a strain of antibiotic–producing *Streptomyces philanthi* within specialized glands on her antenna. *S. philanthi* then excrete antibiotics into the cocoons, protecting the beewolf larvae from harmful pathogens (Kroiss et al. 2010). Bream et al. (2001) investigated the biological activity of the secondary metabolites of 41 Egyptian actinomycete strains on the cotton leaf worm *Spodoptera littoralis*. They found that 58% of the tested strains caused larval mortality ranging from 10-60%; *Streptomyces* and *Streptoverticillium* were the most potent actinomycetes affecting the biological and physiological criteria of the present insect species. At present, microbial insecticides are the main component of the bio-pesticide industry (Shi 2000, Xie 1998).

The main purpose of the agricultural studies is to increase the yield of product per hectare. The primary damage of Colorado potato beetle is leaf feeding by larvae and adults, although young fruits can also be eaten if the host is eggplant or tomato. Leaf feeding has the greatest effect on potato growth if it occurs within two weeks of peak flowering; leaf feeding during the last few weeks before harvest or very early in the growth of the crop has little effect on yield in Turkey. Up to now, chemical substance such as deltamethrin from synthetic pyrethroid group has been utilized to control this pest (Burg et al. 1979).

The purpose of this study was to investigate the insecticidal effects of thirty-seven *Streptomycetes* sp. strains isolated from different soils and geographical areas in Turkey on adults and larvae of *L. decemlineata*.

MATERIAL AND METHOD

Collection of insects

Larvae and adults of Colorado potato beetle were collected from the vicinity of Samsun and Ordu in Turkey from July to August 2004. For this study, larvae and adults were taken from the gardens to the laboratory in appropriate boxes and

reared in groups of 20 larvae and 10 adults in containers. Containers were punched to permit air flow.

Growth of bacteria

Each of *Streptomycetes* strains was received with sterile toothpick from the pure culture stocks (Gürel 2006) and transferred to ISP4 agar in the petri dishes. The strains were grown at 25±2 in ISP4 (8 days culture of sporulation). After incubation, the strains were used for preparation of solutions (A, B, C).

Bioassays

Each group was fed fresh potato plant leaves for 48 hr. For this purpose, diets were placed into plastic containers of 100 mm in diameter for each *Streptomycetes* strains. The surface of the diet in each container was contaminated individually with the agent prepared in PBS using sterilized syringe (Dulmage 1981).

Preparation of solution A

Each of *Streptomycetes* strains was received with sterile toothpick from ISP4 agar and placed into 10 ml sterile glasses. Three milliliters chloroform was added into these glasses. All glasses were shaken during five minutes. The chloroform was dried up in the incubator. Then 8 ml sterile serum physiological was added in to these glasses (Fabre et al. 1988). This solution was used as solution A.

Preparation of solution B

The liquid nutrient (not added agar to ISP4) was prepared into the 10 ml sterile glasses. Each of *Streptomycetes* strains were received with sterile toothpick from the solid nutrient (ISP4) and placed into these glasses. The inoculated glasses were kept on a rotary shaker at 110 rpm at 23±2 ºC for seven days.

The fermentation product of *Streptomycetes* strains was centrifuged at room temperature and $3\text{-}5x10^3$ rpm for 5 minutes to separate the mycelia from the product. Upper side or supernatants were used as solution B.

Preparation of solution C

Each of *Streptomycetes* strains was received from the nutrient (ISP4) and placed into 10 ml sterile glasses. Then 6 ml sterile isotonic serum physiological was added into these glasses. This solution was used as solution C.

Determination of the insecticidal effect of solutions

Healthy third-fourth instars larvae and adults of *L. decemlineata* were used for the insecticidal effect of the each type of *Streptomyces* solutions (A, B, C) used for bio assays. The diet was prepared each type of solutions (A, B, C) and placed into individual glass containers (80 x 100 mm in diameters). Twenty larvae and ten adults were used at each assay.

After 48 hours, the larvae were received fresh diet every 24 hours. Twenty control larvae were received diet contaminated with sterile distilled water for the first 48

hours. Then they were received fresh diet every 24 hours. Finally dead larvae and adults were removed (Thiery and Frachon 1997). All larvae and adults were kept at 26±2 ºC at 60% RH (relative humidity) with a 12:12 hr photoperiod. Dead larvae and adults were removed immediately, and bioassay was checked daily till 8th date. Data were evaluated by using Abbot's formula (Abbott 1925).

RESULTS AND DISCUSSION

There has recently been an increasing interest in finding more effective and safe biological control agents against hazardous insects. In this study, in order to find a more effective and safe pesticide, we tested the insecticidal effects of prepared solutions (A,B,C) from thirty-seven *Streptomycetes* strains on *L. decemlineata* larvae and adults. Treatment mortality was calculated using Abbot's formula.

In this study; it was determined that following eight days of bioassay, larvae showed different signs before death; generally the larvae displayed sluggishness, loss of appetite and depth of color. In addition to it was determined that during bioassays, adults showed signs before death; generally the adults displayed, loss of appetite and depth of color. In bioassays, the highest insecticidal effect determined on *L. decemlineata* larvae within eight days were found as 98.3% for strain M4010; 86.2% for strain M1256; 77.6% for strain M4013, M3024, M2048 and M1483; 67.2% for strain M7008, M1478 and M1328 with solution A (Table 2). In contrast, the insecticidal effect of solution A on *L. decemlineata* adults was determined at lower mortality ranging from 17.9 to 35.9% (Table 1).

Table 1. Insecticidal effects of solution A* on *L. decemlineata* adults

Strain no	Death number					Death rate			Death rate (%) of Abbott
	Min.	Max.	Average death **	±	Standard deviation	%***	±	Standard deviation	
M5099	5	6	5,50	±	0,71	27,50	±	3,54	25,6
M4014	4	4	4,00	±	0,00	20,00	±	0,00	17,9
M4011	7	8	7,50	±	0,71	37,50	±	3,54	35,9
M3024	4	4	4,00	±	0,00	20,00	±	0,00	17,9
M3004	4	4	4,00	±	0,00	20,00	±	0,00	17,9
M2048	5	6	5,50	±	0,71	27,50	±	3,54	25,6
M2033	7	8	7,50	±	0,71	37,50	±	3,54	35,9
M1484	5	6	5,50	±	0,71	27,50	±	3,54	25,6
M1483	7	8	7,50	±	0,71	37,50	±	3,54	35,9
M1389	4	4	4,00	±	0,00	20,00	±	0,00	17,9
M1282	4	4	4,00	±	0,00	20,00	±	0,00	17,9
M1249	4	4	4,00	±	0,00	20,00	±	0,00	17,9

* See material and methods for prepare of solution A

** The average is numbers of death for two groups with twenty adults

*** The average is the rate of death for two groups with twenty adults

Table 2. Insecticidal effects of solution A* on *L. decemlineata* larvae

Strain no	Death number					Death rate			Death rate(%) of Abbott
	Min.	Max.	Average death **	±	Standard deviation	% ***	±	Standard deviation	
M8042	10	12	11,33	±	1,15	56,7	±	5,77	55,17
M8034	11	12	11,33	±	0,58	56,7	±	2,89	55,17
M7008	13	14	13,67	±	0,58	68,3	±	2,89	67,24
M7006	10	12	11,00	±	1,00	55,0	±	5,00	53,45
M7002	10	12	11,00	±	1,00	55,0	±	5,00	53,45
M5099	13	14	13,33	±	0,58	66,7	±	2,89	65,52
M4033	10	12	10,67	±	1,15	53,3	±	5,77	51,72
M4032	11	12	11,33	±	0,58	56,7	±	2,89	55,17
M4013	15	16	15,67	±	0,58	78,3	±	2,89	77,59
M4012	10	12	11,00	±	1,00	55,0	±	5,00	53,45
M4010	19	20	19,67	±	0,58	98,3	±	2,89	98,28
M3024	15	16	15,67	±	0,58	78,3	±	2,89	77,59
M2048	15	16	15,67	±	0,58	78,3	±	2,89	77,59
M1483	15	16	15,67	±	0,58	78,3	±	2,89	77,59
M1478	13	14	13,67	±	0,58	68,3	±	2,89	67,24
M1446	10	12	11,00	±	1,00	55,0	±	5,00	53,45
M1389	5	6	5,67	±	0,58	28,3	±	2,89	25,86
M1329	10	12	11,00	±	1,00	55,0	±	5,00	53,45
M1328	13	14	13,67	±	0,58	68,3	±	2,89	67,24
M1256	17	18	17,33	±	0,58	86,7	±	2,89	86,21
M1249	8	10	9,33	±	1,15	46,7	±	5,77	44,83
M1074	10	12	11,00	±	1,00	55,0	±	5,00	53,45

* See material and methods for prepare of solution A

** The average is numbers of death for two groups with twenty larvae

*** The average is the rate of death for two groups with twenty larvae

The insecticidal effect of solution B of *Streptomycetes* strains determined on *L. decemlineata* larvae was found as 96.6% for strain M3024; 86.2% for strain M5099; 84.5% for strain M1202; 75.9% for strain M1099 (Table 4). In contrast, the insecticidal effect of solution B on *L. decemlineata* adults was determined at lower mortality ranging from 25.6 to 56.4% (Table 3).

Table 3. Insecticidal effects of solution B* on *L. decemlineata* adults

Strain no	Death number					Death rate			Death rate(%) of Abbott
	Min.	Max.	Average death **	±	Standard deviation	% ***	±	Standard deviation	
M8042	5	6	5,50	±	0,71	27,50	±	3,54	25,6
M7002	11	12	11,50	±	0,71	57,50	±	3,54	56,4
M4033	7	8	7,50	±	0,71	37,50	±	3,54	35,9
M4013	5	6	5,50	±	0,71	27,50	±	3,54	25,6
M4011	7	8	7,50	±	0,71	37,50	±	3,54	35,9
M1484	7	8	7,50	±	0,71	37,50	±	3,54	35,9
M1083	7	8	7,50	±	0,71	37,50	±	3,54	35,9

* See material and methods for prepare of solution B.
** The average is numbers of death for two groups with twenty adults
*** The average is the rate of death for two groups with twenty adults

Table 4. Insecticidal effects of solution B* on *L. decemlineata* larvae

Strain no	Death number					Death rate			Death rate(%) of Abbott
	Min.	Max.	Average death **	±	Standard deviation	% ***	±	Standard deviation	
M7005	10	12	11,00	±	1,00	55,00	±	5,00	53,4
M5099	17	18	17,33	±	0,58	86,67	±	2,89	86,2
M5046	12	14	13,00	±	1,00	65,00	±	5,00	63,8
M3024	19	20	19,33	±	0,58	96,67	±	2,89	96,6
M1389	12	14	13,00	±	1,00	65,00	±	5,00	63,8
M1202	16	18	17,00	±	1,00	85,00	±	5,00	84,5
M1099	15	16	15,33	±	0,58	76,67	±	2,89	75,9

* See material and methods for prepare of solution B
** The average is numbers of death for two groups with twenty larvae
*** The average is the rate of death for two groups with twenty larvae

Insecticidal effects of the solution C of *Streptomycetes* strains on *L. decemlineata* adults were shown at Table 5. About three strains caused adult mortality ranging from 30.8 to 33.3%, thirty three strains caused increased larval mortality ranging from 50.00 to 93.1% (Table 6).

Table 5. Insecticidal effects of solution C* on *L. decemlineata* adults

Strain no	Death number					Death rate			Death rate(%) of Abbott
	Min.	Max.	Average death **	±	Standard deviation	% ***	±	Standard deviation	
M4010	**6**	8	**7,00**	±	**1,41**	35,00	±	7,07	33,3
M2033	**5**	6	**5,50**	±	**0,71**	27,50	±	3,54	25,6
M1484	**6**	7	**6,50**	±	**0,71**	32,50	±	3,54	30,8

* See material and methods for prepare of solution C
** The average is numbers of death for two groups with twenty adults
*** The average is the rate of death for two groups with twenty adults

Table 6. Insecticidal effects of solution C* on *L. decemlineata* larvae

Strain no	Death number					Death rate			Death rate(%) of Abbott
	Min.	Max.	Average death**	±	Standard deviation	% ***	±	Standard deviation	
M8042	14	15	14,67	±	0,58	73,33	±	2,89	72,41
M8034	12	13	12,67	±	0,58	63,33	±	2,89	62,07
M7008	14	16	15,00	±	1,00	75,00	±	5,00	74,14
M7006	12	13	12,67	±	0,58	63,33	±	2,89	62,07
M7002	10	11	10,33	±	0,58	51,67	±	2,89	50,00
M5099	15	17	16,67	±	0,58	83,33	±	5,77	82,76
M5046	17	18	17,67	±	0,58	88,33	±	2,89	87,93
M5006	14	15	14,67	±	0,58	73,33	±	2,89	72,41
M4033	14	16	15,00	±	1,00	75,00	±	5,77	74,14
M4032	13	14	13,67	±	0,58	68,33	±	2,89	67,24
M4015	10	12	11,00	±	1,00	55,00	±	5,00	53,45
M4014	13	14	13,33	±	0,58	66,67	±	2,89	65,52
M4013	14	15	15,00	±	1,00	75,00	±	2,89	74,14
M4012	10	11	10,33	±	0,58	51,67	±	2,89	50,00
M4011	16	17	16,67	±	0,58	83,33	±	2,89	82,76
M4010	12	13	12,67	±	0,58	63,33	±	2,89	62,07
M3024	16	17	16,67	±	0,58	83,33	±	2,89	82,76
M3004	13	14	13,67	±	0,58	68,33	±	2,89	67,24
M2048	16	17	16,67	±	0,58	83,33	±	2,89	82,76
M2033	16	17	16,67	±	0,58	83,33	±	2,89	82,76
M1484	10	12	11,00	±	1,00	55,00	±	5,00	53,45
M1483	18	19	18,67	±	0,58	93,33	±	2,89	93,10
M1478	16	17	16,67	±	0,58	83,33	±	2,89	82,76
M1446	14	15	14,67	±	0,58	73,33	±	2,89	72,41
M1389	13	14	13,33	±	0,58	66,67	±	2,89	65,52
M1329	14	16	15,00	±	1,00	75,00	±	5,00	74,14
M1328	14	16	15,00	±	1,00	75,00	±	5,77	74,14
M1282	18	19	18,67	±	0,58	93,33	±	2,89	93,10
M1268	10	12	11,00	±	1,00	55,00	±	5,77	53,45
M1265	10	11	10,33	±	0,58	51,67	±	2,89	50,00
M1202	16	17	16,67	±	0,58	83,33	±	2,89	82,76
M1099	16	17	16,67	±	0,58	83,33	±	2,89	82,76
M1083	14	16	15,00	±	1,00	75,00	±	5,00	74,14

* See material and methods for prepare of solution C

** The average is numbers of death for two groups with twenty larvae

*** The average is the rate of death for two groups with twenty larvae

These results proved the insecticidal effects of the secondary metabolites used similar toxic effects of the secondary metabolites were reported by Fabre et al. (1988) against the housefly *Musca domestica*, by Dindo (1993) against different insect pests, by Vijayan and Balaraman (1991) against mosquito larvae *Culex*

quinquefasciatus and *Anopheles stephenn* and, by Mishra et al. (1987) against *Aedes aegypti*. The present results are, however, in accordance with several results performed with actinomycetes and other insect species. Dhanasekaran et al. (2010) found that the actinomycete isolates producing strong larvicidal activity against *Anopheles* mosquito larvae. A slight larval distortions or abnormalities were recorded. In another study; recently, a highly efficacious protein that kills boll weevil larvae, a key cotton pest, was discovered in S*treptomyces* culture filtrates (Purcell et al. 1993). The protein was identified as cholesterol oxidase. Cholesterol oxidase disrupted the midgut epithelium at lower doses and lysed the midgut cells at higher doses.

According to previous studies, the preliminary bioassays of extracts from a variety of nitrogen-fixing *Streptomyces* spp. obtained from China shown that one of the strains *Streptomyces griseofuscus* (MS/ZD/033), produced the most active metabolite against mosquito larvae *Aedes aegypt* (Zhang et al. 1997). In another study; a culture of *Streptomyces aurantiacus*, producer of aleucide, which is characterized by high insecticide activity, has been isolated in a search for novel natural insecticides (Shopotova et al. 1993). Many actinomycete strains caused larval mortality, of the cotton leaf worm *Spodoptera littoralis*, ranging from 10 to 60% (Bream et al. 2001).

The secondary metabolites of new strain of *Streptomyces* displayed growth inhibition on the test pathogenetic insects, such as *Spodoptera exigua, Dendrolimus punctatus, Plutella xylostella, Aphis glycines* and *Culex pipiens* (Huamei et al. 2008).

Since the cuticle of many insect species consists largely of chitin, it was postulated that chitinase produced by these isolates could be involved in insect control. So, the production of chitinase was used as the criteria for the selection of important biocontrol agents of many insects. Microbial chitinolytic enzymes have been considered properties in the biological control of many insects due to their ability to interfere with chitin deposition (Tripathi et al. 2002). As a result of study, some strains showed higher insecticidal effect, these strains may have chitinase enzyme. Microbial soil cultures were given as food to detect insecticidal produce (Fabre et al. 1988).

In conclusion, we isolated and characterized different *Streptomycetes* from soil. In addition, 37 different *Streptomycetes* species were also tested against *L. decemlineata* larvae and adults. Some of the isolates appear to be significant candidates in biological control of this pest. Especially, 98.3% for strain M4010; 86.2% for strain M1256; 96.6% for strain M3024; 86.2% for strain M5099 and 84.5% for strain M1202 are the most promising ones. This study also shows that adults and larval stages of *L. decemlineata* were susceptible to the isolate 98.3% for strain M4010 (solution A) and 96.6% for strain M3024 (solution B). Adults of *L. decemlineata* were sensitive 56.4% for strain M7002 (solution B). Finally, we

determined that isolates M3024 and M4010 might be especially used in potato fields to control *L. decemlineata* larvae. However the isolate M7002 might be used for *L. decemlineata* adults. However, further research needs to be directed to improve the insecticidal potential of these isolates using recombinant techniques as a biological control agent of *L. decemlineata*.

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