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Original article (Orijinal araştırma)

Determination of insecticide resistance levels of *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) in potato fields of Niğde, Konya, and Afyonkarahisar provinces[1]

Niğde, Konya ve Afyonkarahisar İlleri patates ekim alanlarında *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)'nın insektisitlere direnç seviyelerinin belirlenmesi

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Abstract

In our research, acetamiprid, spinosad, and deltamethrin resistance levels were determined in *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) populations collected from two different locations in potato cultivation areas of Niğde, Afyonkarahisar, and Konya provinces, where 34.8% of the amount was produced. The insecticides were applied using a spray tower at different concentrations. 3-4th instar larvae were subjected to residual effect tests. The susceptible population was obtained from Niğde Ömer Halisdemir University. Field populations were collected starting from June 2023. In the residual effect tests, at the end of the 24-hour exposure period, the LC_{50} values obtained from the susceptible population, Sandıklı, Şuhut, Konaklı, Çiftlik, Çumra, Selçuklu populations were determined as 1.507, 111.964, 89.617, 81.977, 73.563, 38.062, 7.409 mg ai l^{-1} for acetamiprid; 1.105, 21.035, 16.218, 11.982, 15.841, 8.183, 8.945 mg ai l^{-1} for deltamethrin and 4.738, 104.224, 96.404, 70.211, 64.147, 56.863, 37.639 mg ai l^{-1} for spinosad, respectively. The highest resistance rate was detected in the Sandıklı population as 74.30-fold, 19.04-fold, and 22-fold for acetamiprid, deltamethrin, and spinosad, respectively. Considering insecticide resistance, it is important to give priority to insecticides to which the pest has developed less resistance in chemical control of *L. decemlineata*.

Keywords: Acetamiprid, deltamethrin, insecticide resistance, *Leptinotarsa decemlineata*, spinosad

Özet

Araştırmamızda üretiminin %34.8'inin yapıldığı Niğde, Afyonkarahisar ve Konya illeri patates ekim alanlarındaki ikişer farklı lokasyondan toplanan *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) popülasyonlarında acetamiprid, spinosad ve deltamethrin direnç seviyeleri belirlenmiştir. İnsektisitler farklı konsantrasyonlarda püskürtme kulesi kullanılarak uygulanmıştır. 3-4. dönem larvalar rezidüel etki testlerine tabi tutulmuştur. Hassas popülasyon Niğde Ömer Halisdemir Üniversitesinden elde edilmiştir. Arazi popülasyonları 2023 yılı haziran ayından itibaren toplanmıştır. Rezidüel etki testlerinde 24 saat maruz bırakma süresi sonunda hassas popülasyon, Sandıklı, Şuhut, Konaklı, Çiftlik, Çumra, Selçuklu, popülasyonlarından elde edilen LC_{50} değerleri sırasıyla acetamiprid için 1.507, 111.964, 89.617, 81.977, 73.563, 38.062, 7.409 mg ai l^{-1}; deltamethrin için 1.105, 21.035, 16.218, 11.982, 15.841, 8.183, 8.945 mg ai l^{-1} ve spinosad için 4.738, 104.224, 96.404, 70.211, 64.147, 56.863, 37.639 mg ai l^{-1} olarak belirlenmiştir. En yüksek direnç oranı acetamiprid, deltamethrin ve spinosad için sırasıyla 74.30 kat, 19.04 kat, 22 kat olarak Sandıklı popülasyonunda tespit edilmiştir. İnsektisit direnci dikkate alındığında *L. decemlineata* ile kimyasal mücadelede zararlının daha az direnç geliştirdiği insektisitlere öncelik verilmesi önem arz etmektedir.

Anahtar sözcükler: Acetamiprid, deltamethrin, insektisit direnci, *Leptinotarsa decemlineata*, spinosad

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Introduction

Potatoes (*Solanum tuberosum* L. 1753 (Solanales**:** Solanaceae)) rank 4th in agricultural production after maize, rice, and wheat (Kumar & Pandey, 2008). According to FAO reports, approximately 18.2 million hectares of potato were planted worldwide in 2021. Turkey contributes 1.4% of the world's potato production and ranks 16th in terms of production volume (Anonymous, 2023). Potatoes play a significant role in Turkey's economic growth (approximately 3% of Gross Domestic Product) and are the most consumed vegetable in the country (Günel et al., 2010).

In 2022, Turkey's total potato production was 5.200.000 tons, with an average yield of 3.739 kg/da. Niğde leads in potato production with 176.300 da (12.7%) of planting area and 679.653 tons (13.1%) of production. Afyonkarahisar ranks second with 149.968 da (10.8%) of planting area and 607.539 tons (11.7%) of production, while Konya ranks third with 120.491 da (8.7%) of planting area and 518.677 tons (10%) of production (Anonymous, 2023).

In potato cultivation, factors such as insects, diseases, and weeds adversely affect the quality and quantity of the crop. It has been reported that 270 insect and 17 mite species are harmful to potato worldwide (Alkan et al., 2017).

In many parts of the world, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), is a major pest of potato. Yield losses due to this pest range from 30-50% annually, with losses up to 100% in dense populations (Zhou et al., 2012). The leaf area consumed by a single individual over its lifetime can reach 100 cm^2 (Ferro et al., 1985). The most damaging period for the Colorada potato beetle is the mature larval stage, during which 90% of the total leaf consumption occurs (Hare, 1990).

The first record of resistance in the *L. decemlineata* was reported in 1952 for DDT (Quinton, 1955), and later in 1968 for dieldrin (Gauthier et al., 1981). It has been noted that populations susceptible to carbofuran can develop resistance within a single growing season (Ioannidis et al., 1992). Similarly, resistance to endosulfan was detected in the year of its registration (Sharif et al., 2007). The extensive use of chemical control methods in the control of *L. decemlineata* has caused the pest to become resistant to many insecticide classes. This has made resistance management necessary (Gökçe et al., 2018). Today resistance has been observed to 56 different active ingredients from 13 insecticide groups (Özdemir et al., 2021).

In our country, the first study was conducted in 1974-1975. It was found that the *L. decemlineata* did not develop resistance to azinphosmethyl, trichlorphon, and propoxur (Atak & Atak, 1977). This was explained by the pest's recent introduction to the country and limited use of insecticides. In 1980, chemical control was applied to 19% of potato fields, while this rate increased to 54% by 1988 (Ünal & Kılıç, 1997). Resistance to azinphosmethyl was detected in Bolu, Nevşehir, and Tekirdağ populations at 11.24, 8.99, and 9.04-fold, respectively, and for endosulfan and carbofuran at 5.29, 3.82, 6.83-fold, and for deltamethrin at 225.92, 58.83, 90.42-fold, and for endosulfan at 15.24, 17.58, and 45.46-fold (Erdoğan & Gürkan, 1997).

Resistance may be defined as 'a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species' (IRAC 2024). There are 5 types of resistance in harmful insects: behavioral, morphological, physiological, multi-directional and cross-resistance (Yu, 2008). There are some enzymes that are effective when insecticides enter the insect body. These enzymes, such as P450, glutathione-S-transferase (GST) and hydrolase, help eliminate the toxicity of chemicals in insecticides (Tsagkarakou et al., 2009).

The significant procreative potential of *L. decemlineata* accelerates the development of mutations that lead to resistance (Bishop & Grafius, 1996). The beetle, which feeds on Solanaceae family plants, has developed physiological capabilities to detoxify toxic glycoalkaloids found in the leaves (Ferro, 1993). The

L. decemlineata, which can feed on tobacco (*Nicotiana tabacum* L., 1753 (Solanales: Solanaceae)), has developed resistance to neonicotinoids, analogs of nicotine found in this plant. Resistance of 100-fold was detected 2 years after the registration of imidacloprid (Zhao et al., 2000) and soon reached 300-fold (Mota-Sanchez et al., 2006).

Resistance mechanisms in the *L. decemlineata* include increased secretion of esterases, carboxylesterases, and monooxygenases, target site insensitivity, and increased secretion of detoxification enzymes (Clark et al., 2001). The most common resistance mechanism involves monooxygenases, which also play a significant role in neonicotinoid resistance. These insecticides cause desensitization due to mutations in the target AChE receptors (Stanković et al., 2004).

Desensitization is not limited to AChE receptors; mutations in sodium channels, as seen with permethrin, can also affect imidacloprid sensitivity (Tan et al., 2005).

Resistance in pests is determined by proportional increases in LD_{50} or LC_{50} values in laboratory studies. Despite increases in LD_{50} and LC_{50} values, there may not be a noticeable reduction in field control results (Grafius & Douches, 2008). Increases detected in bioassay studies contribute significantly to early warning systems for producers, delay the development of resistance, and support resistance management.

Studies have found that resistance ratios can increase to 220-fold for endosulfan (Sharif et al., 2007), 252-fold for phasolone (Mohamadi et al., 2010), 300- fold for imidacloprid (Mota-Sanchez et al., 2006), and 645-fold for bensultap (Sladan et al., 2012). Resistance ratios also vary regionally and locally (Sharif et al., 2007), depending on application methods and across different pest life stages.

In this study, the resistance ratios of the 3rd and 4th instar larvae of the *L. decemlineata* gathered from the top three potato-producing provinces of Niğde, Afyon, and Konya were determined against acetamiprid, deltamethrin, and spinosad using residual effect tests with different concentrations and exposure durations.

Materials and Methods

Materials

Leptinotarsa decemlineata populations used in the study were collected from potato fields in two locations each in Niğde, Konya, and Afyonkarahisar provinces starting from June 2023 (Table 1). Insects in the larval stage were collected from the fields. The collected larvae were brought to the laboratory and fed on potato leaves in a climate chamber for 24 hours. During this time, individuals damaged during collection and transportation were eliminated (Huseth & Groves, 2013), and healthy 3rd-4th stage larvae were selected and used in the tests. The susceptible population was obtained from the Department of Plant Production and Technology, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University. They were brought to the climate chamber in plastic containers covered with mesh and fed with potato plant leaves that we had previously grown.

Table 1. Provinces and districts where *Leptinotarsa decemlineata* populations were collected

Province	District	Coordinates
Afyon	Şuhut	38°31'03"N 30°32'57"E
	Sandı	38°28'36"N 30°16'12"E
Niğde	Konakl	38°09'34"N 34°49'40"E
	Çiftlik	38°12'26"N 34°31'11"E
Konya	Çumra	37°34'05"N 32°38'33"E
	Selçuk	38°02'02"N 32°30'55"E

A mixture of sandy soil, perlite, and peat was prepared and planted to grow the potato plants. Plantings were made at regular intervals to ensure the continuation of the cultures. The plants and insects were grown in the climate room at 26±1ºC, 60±10% relative humidity, and 16-8 hours (light-dark) photoperiod conditions.

In the study, commercial insecticides often used for *L. decemlineata* control were used: Decis 2.5 EC (containing 2.5% (w/v) deltamethrin from Bayer CropScience), Phomidan 480 SC (containing 480 g/L spinosad from Koruma Tarım) and Goldplan 20 SP (containing 20% acetamiprid from Agrobest Group) (Table 2). A spray tower (Burkard Scientific Laboratory Spray Tower) was used for insecticide applications, and Petri dishes containing larvae were placed in a climate chamber (Nüve TK 120).

Table 2. Information about the insecticides used in the study

Active ingredient name	Trade name	Formulation	Mechanism of Action	Dosage	Action mode	Chemical group
Acetamiprid (% 20)	Goldplan	SP	4A	6 g/da	Nicotinic acetylcholine receptor agonists/antagonists	Neonicotinoids
Deltamethrin (25 g/l)	Decis	EC	3A	30 ml/da	Sodium channel modulators	Pyrethroids, Pyrethrins
Spinosad (480g/L)	Phomidan	SC	5	10 ml/da	Nicotinic Acetylcholine receptor agonists	Spinosyns

Methods

Bioassays

The dose determination study was conducted using a reference susceptible population. Residual effect tests were performed using 3rd-4th instar larvae of *L. decemlineata* Six effective doses causing mortality ranging from 15% to 99% were selected for each active substance in preliminary experiments.

Insecticide concentrations were prepared by diluting with distilled water 50% at each step. Distilled water was used as a control. The prepared insecticides were sprayed into petri dishes (2 ml) using spray tower at 1-atmosphere pressure.

The petri dish (12 cm in diameter, 2.5 cm in height) was left to dry 5 minutes before ten healthy 3rd-4th instar larvae were placed in each using soft forceps (according to the contact and dry film method) (Gokulakrishnaa & Thirunavukkarasu, 2023). No food was added to the petri dishes. The dishes were closed with lid. To prevent larvae from dying due to lack of air, a small rectangular piece of paper was placed between the Petri dish and its lid to avoid the lid closing completely. The experiments were carried out in the climate chamber under conditions of 26±1ºC warmth, 60±10% proportional humidity, and 16-8 hours (light-dark) photoperiod. After 24, 48, and 72 hours of exposure, larvae were touched with a soft brush on various parts of their bodies, and immobile larvae were considered dead (Alkan et al., 2017). Experiments were conducted in a completely randomized design with three replications. One Petri dish was used for each replication.

Data Analysis

The data were subjected to probit analysis, and LC_{50} value was determined using the POLO computer package program (LeOra, 2002). The resistance ratio (RR) was calculated by dividing the LC_{50} values of the resistant group by the LC_{50} values of the susceptible group. Resistance levels were assessed using the scale suggested by Lee et al. (1999): RR <2: Not or minimal, RR = 2-5: Little , RR = 5-10: Medium, RR >10: High resistance.

Results and Discussion

Rate of acetamiprid resistance

The highest resistance rates in the 24-hour exposure period were found to be 74.30-fold in the Sandıklı population, 59.47-fold in the Şuhut population, 54.40-fold in the Konaklı population, 48.81-fold in the Çiftlik population, 31.46-fold in the Selçuklu population and 25.26-fold in the Çumra population, respectively (Table 3). The highest LC_{50} values were found in the Sandıklı population: 111.964 mg ai l^{-1} at 24 hours, 68.804 mg ai l^{-1} at 48 hours, and 29.080 mg ai l^{-1} at 72 hours (Tables 3, 4 & 5).

Table 3. Residual toxicity of acetamiprid to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 24-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	1.507 1.045-2.280	1.255± 0.209	13.212	16	0.826	0.830	-
Sandıklı	180	111.964 73.563-177.976	1.414±0.216	21.131	16	1.320	0.470	74.30
Şuhut	180	89.617 61.602-129.358	1.296±0.210	7.547	16	0.472	0.512	59.47
Konaklı	180	81.977 55.312-118.474	1.268±0.210	10.058	16	0.629	0.528	54.40
Çiftlik	180	73.563 50.951-102.649	1.404 ± 0.218	10.325	16	0.645	0.551	48.81
Çumra	180	38.062 26.783-52.654	1.454±0.219	6.193	16	0.387	0.545	25.26
Selçuklu	180	47.409 34.046-66.186	1.461±0.218	5.841	16	0.365	0.502	31.46

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Table 4. Residual toxicity of acetamiprid to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 48-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	0.856 0.548-1.258	1.184±0.207	7.241	16	0.453	0.558	-
Sandıklı	180	68.804 44.529-100.427	1.209±0.209	15.250	16	0.953	0.558	80.38
Şuhut	180	58.710 36.444-85.991	1.190±0.209	7.948	16	0.497	0.584	68.59
Konaklı	180	38.609 21.768-57.238	1.359±0.235	8.917	16	0.557	0.678	45.10
Çiftlik	180	37.619 22.034-54.608	1.465±0.246	5.876	16	0.367	0.689	43.95
Çumra	180	27.318 17.203-39.101	1.468± 0.238	7.567	16	0.473	0.622	31.91
Selçuklu	180	29.324 17.668-43.191	1.367±0.235	6.962	16	0.435	0.607	34.26

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Similar studies have shown that the *L. decemlineata* has developed varying resistance levels to imidacloprid, a neonicotinoid insecticides. Baker et al. (2014) detected imidacloprid resistance in *L. decemlineata* larvae in three divergent populations in the USA, ranging from 7.6 to 71-fold. In a study conducted between 1995 and 1998 across the United States, Canada, Germany, France, and Poland, the lowest resistance to imidacloprid was reported as 29-fold (Olson et al., 2000). Crossley & Rondon (2018) reported a resistance increase of 9-340-fold among populations in 10 different regions of the USA. Keskin & Yorulmaz Salman (2020) found that populations from the districts of Afyonkarahisar displayed resistance to imidacloprid ranging from 3.96 to 27.31 times. In our study, resistance levels of 59.47-fold and 74.30-fold were determined in Şuhut and Sandıklı districts of Afyonkarahisar, respectively. Given the time elapsed and cross-resistance, it is normal to observe increased resistance in pests due to intensive insecticide applications (both in terms of number of spraying and dosage). It is known that insecticide sensitivity in *L. decemlineata* is highest in young larvae, decreases in mature larvae, and is lowest in adults (Sayıncı et al., 2013). The resistance rates for imidacloprid in Çumra, Karapınar and Seydişehir, Güneysınır, Doğanhisar populations were determined as 4.63, 7.01, 4.16, 2.84 and 2.44-fold, respectively in 2020 (Çağırgan & Çetin, 2021). They reported that imidacloprid was used for treating tubers, and during the growing season, green part applications were also made with imidacloprid (EC formulations) or acetamiprid from the same group. In a study conducted in Serbia, imidacloprid resistance in adult *L. decemlineata* was reported as 82.90-fold (Sladan et al., 2012), while a study in New York reported 29-fold resistance to acetamiprid in adults (Mota Sanchez et al., 2006). Jeschke & Nauen (2008) noted that cross-resistance developed to other neonicotinoid insecticides when one neonicotinoid was used. In our study, the highest resistance ratio was determined for acetamiprid. Considering that neonicotinoids are frequently used for both seed and green part spraying in the control of *L. decemlineata*, acetamiprid resistance is expected to be high.

Table 5. Residual toxicity of acetamiprid to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 72-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	0.461 0.217-0.736	1.132±0.225	10.065	16	0.629	0.668	-
Sandıklı	180	29.080 13.535-45.951	1.225±0.236	5.001	16	0.313	0.712	63.08
Şuhut	180	27.012 12.203-43.035	1.226±0.237	10.491	16	0.656	0.723	58.59
Konaklı	180	23.708 12.502-35.175	1.566±0.279	4.181	16	0.261	0.772	51.43
Çiftlik	180	22.168 11.367-33.110	1.578±0.287	4.946	16	0.309	0.783	48.09
Çumra	180	18.125 9.762-27.302	1.460±0.259	4.995	16	0.312	0.706	39.32
Selçuklu	180	16.733 9.020-25.065	1.515±0.268	2.618	16	0.164	0.723	36.30

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: Degrees of Freedom; H: heterogeneity; RR: resistance rate.

Probit analysis indicates that populations with a slope value less than 2 are heterogeneous (Yu, 2015) and that resistance development is faster in heterogeneous populations (Keskin & Yorulmaz-Salman, 2020). Table 3 shows that heterogeneity values are less than 2.

Rate of deltamethrin resistance

The highest resistance rates in the 24-hour period were determined as 19.04-fold in the Sandıklı population, 14.68-fold in the Şuhut population, 14.34-fold in the Çiftlik population, 10.84-fold in the Konaklı population, 8.10-fold in the Selçuklu population and 7.41-fold in the Çumra population, respectively (Table 6). The highest LC_{50} values were found in the Sandıklı population: 21.035 mg ai l^{-1} at 24 hours, 12.177 mg ai l^{-1} at 48 hours, and 4.492 mg ai l^{-1} at 72 hours (Tables 6, 7 & 8).

Table 6. Residual toxicity of deltamethrin to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 24-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	1.105 0.768-1.713	1.255±0.210	12.144	16	0.759	0.431	-
Sandıklı	180	21.035 14.097-32.557	1.153±0.205	5.303	16	0.331	0.474	19.04
Şuhut	180	16.218 10.772-24.023	1.196±0.206	9.730	16	0.608	0.518	14.68
Konaklı	180	11.982 7.206-18.044	1.098±0.204	11.482	16	0.718	0.566	10.84
Çiftlik	180	15.841 10.917-22.611	1.326±0.213	10.342	16	0.646	0.523	14.34
Çumra	180	8.183 5.729-11.566	1.376±0.214	5.051	16	0.316	0.518	7.41
Selçuklu	180	8.945 6.249-12.823	1.339±0.212	3.571	16	0.223	0.501	8.10

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Table 7. Residual toxicity of deltamethrin to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 48-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	0.470 0.274-0.718	1.057±0.204	12.372	16	0.773	0.574	-
Sandıklı	180	12.177 6.754-19.442	0.954±0.199	9.012	16	0.563	0.557	25.91
Şuhut	180	8.829 4.545-13.869	0.997±0.203	5.533	16	0.346	0.607	18.79
Konaklı	180	6.288 3.243-9.624	1.277±0.232	4.536	16	0.283	0.694	13.38
Çiftlik	180	6.434 3.287-9.898	1.256±0.231	9.004	16	0.563	0.689	13.69
Çumra	180	4.531 2.394-7.046	1.164±0.221	6.118	16	0.382	0.629	9.64
Selçuklu	180	5.209 3.168-7.604	1.403±0.237	4.262	16	0.266	0.618	11.08

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Table 8. Residual toxicity of deltamethrin to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 72-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	0.280 0.158-0.413	1.435±0.249	8.600	16	0.538	0.696	-
Sandıklı	180	4.492 1.442-7.951	0.994±0.224	10.535	16	0.658	0.712	16.04
Şuhut	180	4.057 1.268-7.215	1.021±0.228	9.538	16	0.596	0.729	14.49
Konaklı	180	4.188 2.028-6.422	1.454±0.269	7.104	16	0.444	0.772	14.96
Çiftlik	180	4.073 1.966-6.245	1.472±0.273	10.984	16	0.687	0.778	14.55
Çumra	180	2.382 0.947-3.972	1.208±0.247	7.407	16	0.463	0.739	8.51
Selçuklu	180	2.264 1.124-3.443	1.575±0.294	4.060	16	0.254	0.779	8.09

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Keskin & Yorulmaz Salman (2020) reported that populations from Afyonkarahisar's districts exhibited 9.41-77.17-fold resistance to deltamethrin. Sladan et al. (2012) found up to 60-fold resistance to cypermethrin in adult *L. decemlineata* in Serbia. In a study investigating deltamethrin resistance in 4th instar larvae of *L. decemlineata* collected from 4 different populations from Urumqi, Changji, Qitai and Qapqal (China) in 2009 and 2010, 1.7- to 42.7-fold resistance to deltamethrin was detected (Jiang et al., 2011). Jörg et al. (2007) reported that the effectiveness of lambda-cyhalothrin decreased from 92% in 2003 to 73% in 2006 due to sequential applications. Sladan et al. (2012) found up to 60-fold resistance to cypermethrin, another pyrethroid, in a study conducted in Serbia. Erdoğan & Gürkan (1997) found resistance levels to deltamethrin in *L. decemlineata* adults and 3rd instar larvae to be 225.92, 58.83, and 90.42-fold for Bolu, Nevşehir, and Tekirdağ provinces, respectively. Resistance rates for lambda-cyhalothrin in the Çumra, Karapınar and Seydişehir populations were determined as 2.98, 2.63 and 2.40-fold, respectively in 2020 (Çağırgan & Çetin, 2021). In our study, resistance ratios to deltamethrin in six populations ranged from 7.41 to 19.04-fold. These resistance ratios are lower compared to other studies.

Rate of spinosad resistance

The highest resistance rates in the 24-hour period were determined as 22-fold in the Sandıklı population, 20.35-fold in the Şuhut population, 14.82-fold in the Konaklı population, 13.54-fold in the Çiftlik population, 12-fold in the Çumra population, and 7.94-fold in the Selçuklu population, respectively (Table 9). The highest LC_{50} values were found in the Sandıklı population: 104.224 mg ai l^{-1} at 24 hours, 56.777 mg ai l^{-1} at 48 hours, and 34.410 mg ai l^{-1} at 72 hours (Tables 9, 10 & 11).

Table 9. Residual toxicity of spinosad to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 24-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	4.738 3.274-6.877	1.294±0.210	8.223	16	0.514	0.502	-
Sandıklı	180	104.224 71.255-150.526	1.286±0.210	8.915	16	0.557	0.518	22.00
Şuhut	180	96.404 60.802-147.107	1.091±0.202	7.464	16	0.466	0.529	20.35
Konaklı	180	70.211 45.000-100.637	1.275±0.213	10.978	16	0.686	0.589	14.82
Çiftlik	180	64.147 42.359-89.534	1.408± 0.222	8.356	16	0.522	0.611	13.54
Çumra	180	56.863 40.130-80.802	1.379±0.213	4.578	16	0.286	0.502	12.00
Selçuklu	180	37.639 26.212-51.535	1.502±0.223	2.274	16	0.142	0.584	7.94

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Table 10. Residual toxicity of spinosad to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 48-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	2.501 1.454-3.732	1.136±0.210	13.952	16	0.872	0.608	-
Sandıklı	180	56.777 33.961-82.914	1.225±0.214	7.983	16	0.499	0.623	22.70
Şuhut	180	41.753 21.667-63.596	1.159±0.216	3.713	16	0.232	0.667	16.69
Konaklı	180	35.475 16.974-55.507	1.249±0.237	9.628	16	0.602	0.712	14.18
Çiftlik	180	34.077 19.996-48.610	1.682±0.283	5.693	16	0.356	0.751	13.63
Çumra	180	29.907 17.665-44.091	1.346±0.230	3.290	16	0.206	0.634	11.96
Selçuklu	180	26.615 16.849-37.480	1.607±0.253	4.540	16	0.284	0.667	10.64

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Table 11. Residual toxicity of spinosad to 3rd-4th instar larvae of *Leptinotarsa decemlineata* after a 72-hour exposure period

Population	n	LC_{50} (mg ai l^{-1}) (%95 CL)	Slope ± SEM	λ^2	DF	H	p value	RR
Susceptible	180	1.605 0.795-2.484	1.272±0.240	9.679	16	0.605	0.702	-
Sandıklı	180	34.410 16.332-53.906	1.262±0.240	9.504	16	0.594	0.718	21.44
Şuhut	180	25.375 10.430-41.368	1.260±0.250	4.774	16	0.298	0.761	15.81
Konaklı	180	26.067 12.577-39.967	1.472±0.273	3.782	16	0.236	0.778	16.24
Çiftlik	180	24.119 12.742-35.244	1.764±0.324	5.470	16	0.342	0.811	15.03
Çumra	180	18.313 9.136-28.341	1.384±0.253	3.581	16	0.224	0.728	11.41
Selçuklu	180	14.043 7.654-20.321	1.866±0.347	4.549	16	0.284	0.801	8.75

n: the count of larvae; SEM.: standart error of the mean; λ^2: chi square; DF: degrees of freedom; H: heterogeneity; RR: resistance rate.

Sayıncı et al. (2013) reported that in Erzurum, *L. decemlineata* larvae and adults showed increased resistance to spinosad as their developmental stage progressed. They found that spinosad had 93.3% effectiveness on 2nd instar larvae and 50% effectiveness on 4th instar larvae and adults after 72 hours of application in the laboratory. Çağırgan & Çetin (2021) found spinosad effectiveness to be 75.33% and 76.05% during the first applications in 2019 and 2020, respectively. Osman (2010) noted that in Russia, the effectiveness of spinosad on mature larvae increased over time, with mortality rates rising from 57.78%

on the 3rd day to 95.56% on the 7th day. Previous studies have reported that spinosad's mode of action is both through ingestion and contact (Kowalska, 2010), with effects beginning to be noticeable 4-5 hours after application (Azimi et al., 2009) and providing high protection for up to 20 days (Igrc et al., 1999). In Croatia, spinosad's effectiveness on larvae was reported as 99.9% on the 2nd day, 99.7% on the 7th day, 98.4% on the 14th day, and decreasing to 80.6% by the 21st day (Igrc et al., 1999). Mota-Sanchez et al. (2006) found up to 7.6-fold resistance in adults to spinosad. In the USA, research on four different populations of *L. decemlineata* larvae showed spinosad resistance ranging from 17.5 to 40.6-fold (Schnaars-Uvino & Baker, 2021). Klein (2019) detected spinosad resistance in 2nd instar larvae of three different *L. decemlineata* populations in Long Island (USA). In this study, two susceptible populations, one reared in a laboratory and one collected from organic fields, were used. When the laboratory susceptible population was used, the resistance rates were found to be 52.4, 38.43 and 33.99 times, and when the field susceptible population was used, the resistance rates were 213.33, 156.46 and 138.37 times. These results showed that the resistance rates were higher when the laboratory-reared susceptible population was used and lower when the field-collected susceptible population was used. In our study, spinosad resistance rates in *L. decemlineata* larvae ranged from 7.94 to 22.00 times.

In the context of potato production in Turkey, insecticides have been ranked as follows based on resistance levels in the Niğde, Afyonkarahisar, and Konya populations: acetamiprid, deltamethrin, and spinosad. This ranking is consistent with the frequency and dose of insecticide applications during the potato production season.

Compared to deltamethrin and spinosad, the higher resistance to acetamiprid is due to the use of neonicotinoids in both foliar and tuber applications, which promote cross-resistance and increase resistance to acetamiprid. Monitoring the number of insecticides used, their doses (especially when recommended doses are exceeded) and their frequency is essential for effective pest control. Continuous monitoring of resistance levels in *L. decemlineata* and updating pest management programs are vital to improving the effectiveness of chemical control. Detailed studies of the annual increase in resistance and cross-resistance are also essential.

References

Alkan, M., A. Gökçe & K. Kara, 2017. Stomach poison activity of some plant extracts on Colorado potato beetle (Coleoptera: Chrysomelidae). Plant Protection Bulletin, 57 (3): 305-315.

Anonymous, 2023. Patates ürün raporu 2023.Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü. (tarimorman.gov.tr) (Access date:10 Ocak 2024).

Atak,E.D, & U. Atak,1977. Marmara bölgesinde Patates böceği (*Leptinotarsa decemlineata* Say)'nin insektisitlere karşı direnci üzerinde çalışmalar. Bitki Koruma Bülteni 17 (1): 29-40 (in Turkish).

Azimi, M., A. Pourmirza, M. Safaralizadeh, & G. Mohitazar, 2009. Studies on the lethal effects of spinosad on adults of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) with two bioassay methods. Asian Journal of Biological Sciences, 2 (1): 1-6.

Baker, M., K. Hossain, K. Zabierek, K. Collie, A. Alyokhin & A. Mota-Sanchez, 2014. Geographicvariation in cannibalism in Colorado potato beetle (Coleoptera: Chrysomelidae) populations. Environmental Entomology, 43 (1): 102 109.

Bishop, B. A. & E. Grafius,1996. “Insecticide Resistance in the Colorado Potato Beetle, 355-377”. In Chrysomelidae Biology (Eds. P. Jolivet & T. H. Hsio). SBP Academic Publishing, Amsterdam, 465 pp.

Çağırgan, O. & H. Çetin, 2021. Determination of lambda-cyhalothrin and imidacloprid resistance and synergistic activity of diatomaceous earth in *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) populations. Turkish Journal of Entomology, 45 (4): 475-483.

Clark, J. M., S. H. Lee, H. J. Kim, K. S. Yoon & A. G. Zhang, 2001. DNA-based genotyping techniques for the detection of point mutations associated with insecticide resistance in Colorado potato beetle *Leptinotarsa decemlineata*. Pest Management Science, 57 (10): 968-974.

Crossley, M. S., S. I. Rondon & S. D. Schoville, 2018. A comparison of resistance to imidacloprid in Colorado potato beetle (*Leptinotarsa decemlineata* Say) populations collected in the Northwest and Midwest U.S. American Journal of Potato Research, 95 (5): 495-503.

Erdoğan, C. & M. Gürkan, 1997. *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)'nın değişik popülasyonlarının, bazı insektisitlere karşı duyarlılığının belirlenmesi üzerinde araştırmalar. Türkiye Entomoloji Dergisi, 21 (4): 299-309 (in Turkish with abstract in English).

Ferro, D. N., 1993. Potential for resistance to *Bacillus thuringiensis*: Colorado potato beetle (Coleoptera: Chrysomelidae) a model system. American Entomologist, 39 (1): 38-44.

Ferro, D. N., J. A. Logan, R. H. Voss & J. S. Elkinton, 1985. Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feding rates. Environmental Entomology, 14 (3): 343-348.

Gauthier, N. L., R. N. Hofmaster & M. Semel, 1981." History of Colorado Potato Beetle Control,13-33". In: Advances in Potato Pest Management (Eds. J. H. Lashomb & R. Casagrande). Hutchinson Ross Publishing Company, Stroudsburg, PA, 289 pp.

Gokulakrishnaa, R. K. & S. Thirunavukkarasu. 2023. Bioassay techniques in entomological research. International Journal of Plant & Soil Science, 35 (16): 363-73.

Gökçe, A., G.V. Bingham & M. E. Whalon, 2018. Impact of long-lasting insecticide-incorporated screens on Colorado potato beetle and plum curculio. Turkish Journal of Agriculture and Forestry, 42 (1): 38-44.

Grafius, E. J. & D. S. Douches, 2008. "The Present and Future Role of Insect-Resistant Genetically Modified Potato Cultivars in IPM, 195-221". In: Integration of Insect-Resistant Genetically Modified Crops within IPM Programs (Eds. J. Romeis, A. M. Shelton, and G. G. Kennedy) Springer Science + Business Media B.V., 441 pp.

Günel, E., M. E. Çalışkan, N. Kuşman, K. M. Tugrul, T. A. Yılmaz, T. Ağırnasligil & H. Onaran, 2010. "Nişasta ve şeker bitkileri üretimi, 377-396". Türkiye Ziraat Mühendisliği VII. Teknik Kongresi (11-15 Ocak 2010 Ankara, Türkiye), TMMOB Ziraat Mühendisleri Odası, 578 s (in Turkish).

Hare, J. D., 1990. Ecology and management of the Colorado potato beetle. Annual Review of Entomology, 35 (1): 81-100.

Huseth, A. S. & R. L. Groves, 2013. Effect of insecticide management history on emergence phenology and neonicotinoid resistance in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Journal of Economic Entomolgy, 106 (6): 2491-2505.

Igrc, J., J. Barčič, R. Dobrinčič & M. Maceljski, 1999. Effect of insecticides on the Colorado potato beetles resistant to OP, OC and P insecticides. Journal of Pest Science, 72 (3): 76-80.

Ioannidis, P. M., E. J. Grafius, J. M. Wierenga, M. E. Whalon & R. M. Hollingworth, 1992. Selection, inheritance and characterization of carbofuran resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae). Pesticide Science, 35 (3): 215-222.

IRAC, 2024. Introduction to Resistance, Insecticide Resistance Action Committee. (Web page: https://irac-online.org/training-centre/resistance/) (Date accessed: 08.08.2024).

Jeschke, P. & R. Nauen, 2008. Neonicotinoids-from zero to hero in insecticide chemistry. Pest Management Science, 64 (11): 1084-1098.

Jiang, W. H., W. C. Guo, W. P. Lu, X. .O. Shi, M. H. Xiong, Z. T. Wang & G. Q. Li, 2011. Target site insensitivity mutations in the AChE and LdVssc1 confer resistance to pyrethroids and carbamates in *Leptinotarsa decemlineata* in northern Xinjiang Uygur autonomous region. Pesticide Biochemistry and Physiology, 100 (1): 74-81.

Jörg, E., K. Falke, P. Racca & P. Węgorek, 2007. Insecticide resistance in Colorado beetles: no let up. Kartoffelbau, (5): 168-173.

Keskin, C. & S. Yorulmaz Salman, 2020. Deltamethrin and Imidacloprid resistance levels of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) populations collected from Afyonkarahisar, Turkey. Turkish Journal of Science and Engineering, 2 (1): 1-5.

Klein, C. M., 2019. The evolution of spinosad resistance in Colorado Potato Beetles (*Leptinotarsa decemlineata*). (Web page: https://academicworks.cuny.edu/gc_etds/3464/) (Date accessed: 29.08.2024).

Kowalska, J., 2010. Spinosad effectively controls Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) in organic potato. Acta Agriculturae Scandinavica, Section B. Soil & Plant Science, 60 (3): 283-286.

Kumar, A. & S. K. Pandey, 2008. Potato production: Harbinger of agricultural sustainability. Indian Farming, 58 (9): 3-7.

Lee, C. Y., L. C. Lee, B. H. Ang & N. L. Chong, 1999. ‘’Insecticide resistance in the *Blattella germanica* (L.) (Dictyoptera: Blattellidae) from hotels and restaurants in Malaysia, 171-182’’. Proceeding of the 3rd International Conference on Urban Pests (1999, Hronov, CzechRepublic), GrafickeZavody, 678 pp.

LeOra, 2002. LeOra Software, Polo-Pc: Probit and Logit Analysis. Berkeley, CA, the USA.

Mohamadi, M. M., M. Mossadegh, M. Hejazi, M. Goodarzi, M. Khanjani & H. Galehdari, 2010. Synergism of resistance to phosalone and comparison of kinetic properties of acetylcholinesterase from four field populations and a susceptible strain of Colorado potato beetle. Pesticide Biochemistry and Physiology, 98 (2): 254-262.

Mota-Sanchez, D., R. M. Hollingworth, E. J. Grafius & D. D. Moyer, 2006. Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Pest Management Science, 62 (1): 30-37.

Olson, E. R., G. P. Dively & J. O. Nelson, 2000. Susceptibility to imidacloprid and cros resistance patterns in Colorado potato beetle (Coleoptera: Chrysomelidae) populations. Journal of Economic Entomology, 93 (2): 447-458.

Osman, M. A. M., 2010. Biological efficacy of some biorational and conventional insecticides in the control of different stages of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Plant Protection Science, 46 (3): 123-134.

Özdemir, E., E. Inak, E. Evlice, E. Yüksel, R. A. Delialioğlu & I. A. Susurluk, 2021. Effects of insecticides and synergistic chemicals on the efficacy of the entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) against *Leptinotarsa decemlineata*. Crop Protection, 144 (6): 105605.

Quinton, R., 1955. DDT-resistant Colorado potato beetles, proceedings of the North Central. Entomological Society of America, 9 (3): 94-95.

Sayıncı, B., S. Bastaban & E. Yıldırım, 2013. “Düşük spinosad dozlarının *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) üzerindeki etkileri, 165-175”. I. Bitki Koruma Ürünleri ve Makineleri Kongresi (2-5 Nisan 2013, Antalya, Türkiye), Ankara GıdaTarım ve Hayvancılık Bakanlığı, Gıda ve Kontrol Genel Müdürlüğü 248 s (in Turkish).

Schnaars-Uvino, K. & M.B. Baker, 2021. High-level field-evolved resistance to spinosad in Colorado potato beetle, *Leptinotarsa decemlineata*, in organically managed fields. Pest Management Science, 77 (10): 4393-4399.

Sharif, M. M., M. J. Hejazi, A. Mohammadi & M. R. Rashidi, 2007. Resistance status of the Colorado potato beetle, *Leptinotarsa decemlineata*, to endosulfan in East Azerbaijan and Ardabil Provinces of Iran. Journal of Insect Science, 7 (31): 1-7.

Sladan, S., K. Miroslav, S. Ivan, J. Snežana, K. Petar, T. Goran & R. Jevdović, 2012. Resistance of Colorado potato beetle (Coleoptera: Chrysomelidae) to neonicotinoids, pyrethroids and nereistoxins in Serbia. Romanian Biotechnological Letters, 17 (5): 7599-7609.

Stanković, S., A. Zabel, M. Kostic, B. Manojlovic & S. Rajkovic, 2004. Colorado potato beetle *Leptinotarsa decemlineata* (Say) resistance to organophosphates and carbamates in Serbia. Journal of Pest Science, 77 (1): 11-15.

Tan, J., Z. Liu, R. Wang, Z. Y. Huang, A. C. Chen, M. Gurevitz & K. Dong, 2005. Identification of amino acid residues in the insect sodium channel critical for pyrethroid binding. Molecular Pharmacology, 67 (2): 513-522.

Tsagkarakou, A., T. V. Leeuwen, A. Khajehalit, M. Grispou, M. S. Williamsons, L. Tirry & J. Vontas, 2009. Identification of pyrethroid resistance associated mutations in the para sodium channel of the two spotted spider mite *Tetranychus urticae* (Acari:Tetranychidae). Insect Molecular Biology, 18 (5): 583-593.

Ünal, G. & K. B. Kılıç, 1997. Patates böceği (*Leptinotarsa decemlineata* Say)'nin mücadelesinde yaygın olarak kullanılan insektisitler ile *Bacillus thuringiensis* var. *tenebrionis*'in entegrasyon olanakları üzerinde toksikolojik çalışmalar. Bitki Koruma Bülteni, 37 (1-2): 67-78 (in Turkish with abstract in English).

Yu, S. J., 2008. The Toxicology and Biochemistry of Insecticides. CRC Pres Taylor Francis Group, 250 pp.

Yu, S. J., 2015. The Toxicology and Biochemistry of Insecticides. CRC Press Taylor & Francis Group 6000 Broken Sound Parkway NW, Suite 300 Boca Raton, 296 pp.

Zhao, J. Z., B. A. Bishop & E. J. Grafius, 2000. Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology, 93 (5): 1508-1514.

Zhou, Z., J. Pang, W. Guo, N. Zhong, Y. Tian, G. Xia & J. Wu, 2012. Evaluation of the resistance of transgenic potato plants expressing various levels of Cry3A against the Colorado potato beetle (*Leptinotarsa decemlineata* Say) in the laboratory and field. Pest Management Science, 68 (12): 1595-1604.