Original article (Orijinal araştırma)

Susceptibility of *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae) to some commonly used insecticides

Chilo partellus Swinhoe, 1885 (Lepidoptera: Crambidae)'un yaygın olarak kullanılan insektisitlere duyarlılığı

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Abstract

Chilo partellus Swinhoe, 1885 (Lepidoptera: Crambidae), a destructive pest of maize, was recently recorded in the Mediterranean Region of Turkey. The pest is considered to be an invasive species, displacing indigenous stem borers in many parts of the world. The aim of the study was to determine the effect of commonly used insecticides in a maize production system in Turkey on egg hatch and mortality of first instar larvae of *C. partellus* under laboratory conditions (27±2ºC, 70% RH and 14:10 h L:D photoperiod) at the Plant Protection Department, Faculty of Agriculture, University of Çukurova. Eight insecticides registered for indigenous lepidopteran pests were used at the recommended rates. The percentage of hatched egg masses were significantly different. The smallest percentage was 30.6% with deltamethrin. The percentages of hatched egg masses were greater than 80% for all other insecticides. Mortality of hatched larvae was significantly different. The highest and lowest mortalities of hatched larvae were 84.5% and 38.2% with emamectin-benzoate and lambda-cyhalothrin, respectively. Seventy-two h after exposure of the first instar larvae to sprayed maize leaf disks, the lowest and highest mortalities were 62.6% and 96.7% with indoxacarb and emamectin-benzoate, respectively. Survival analyses revealed that hazard ratios ranged from 4.91 (95% CI: 1.66-14.6) to 15.6 (95% CI: 5.33-45.6) with chlorpyrifos-ethyl and emamectin-benzoate, respectively. The mortality of first instar larvae was about 16, 10 and 9 times that of the control with emamectin-benzoate, lambda-cyhalothrin and deltamethrin, respectively. Feeding activity of larval stage was reduced by all treatments. The implications of this study are discussed.

Keywords: Black-head stage, *Chilo partellus*, hatch, mortality, survival, Turkey

Öz

Chilo partellus Swinhoe, 1885 (Lepidoptera: Crambidae), Türkiye'de Akdeniz Bölgesi'nde son zamanlarda saptanan oldukça zararlı bir mısır zararlısıdır. İstilacı bir tür olarak değerlendirilen *C. partellus*, dünyanın birçok bölgesinde diğer yerli mısırkurtlarının yerini almaktadır. Bu amaçla, Türkiye'de mısır üretim alanlarında diğer Lepidopter türlerine karşı yaygın olarak kullanılan 8 insektisidin önerilen dozlarının *C. partellus*'un yumurta ve birinci dönem larvalarına etkisi, Çukurova Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü laboratuvarında 27±2ºC, %70 orantılı nem, 14:10 (A: K) koşullarda araştırılmıştır. Bu çalışmayla insektisitlerin yumurtaların açılma oranları ve larva ölüm oranları üzerinde etkili olduğu bulunmuştur. En düşük açılma oranı deltamethrinde %30.6 olarak belirlenmiştir. Diğer tüm insektisit uygulamalarında yumurta açılma oranları %80'nin üzerinde saptanmıştır. Larvaların ölüm oranlarında ise en yüksek oran %84.5 ile emamectin-benzoat'ta belirlenirken, en düşük oran %38.2 ile lambda-cyhalothrinde belirlenmiştir. İnsektisite daldırılmış mısır yaprak disklerinde ise birinci dönem larvaların en düşük ve en yüksek ölüm oranları, 72 saat sonra indoxacarb ve emamectin-benzoat için sırasıyla %62.6 ve % 96.7 olmuştur. Hayatta kalma analizleri, risk oranlarının chlorpyrifos-ethyl ve emamectin-benzoat için sırasıyla 4.9 (%95 CI: 1.66-14.6) ile 15.6 (%95 CI: 5.33-45.6) arasında değiştiğini göstermiştir. Birinci dönem larvalarının ölüm oranlarının kontrol ile kıyaslandığında emamectin-benzoate, lambda-cyhalothrin ve deltamethrinde sırasıyla 16, 10 ve 9 kat yüksek olduğu belirlenmiştir. Ayrıca tüm uygulamalarda larvaların davranışlarında azalma gözlenmiştir.

Anahtar sözcükler: Siyah-baş dönemi, *Chilo partellus*, yumurta açılma, ölüm oranı, yaşam, Türkiye

Introduction

The maize spotted stem borer, *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae), originally from the Indian subcontinent (Harris, 1989) has been reported in many parts of the world especially in Asia, eastern and southern parts of Africa (Ndema et al., 2001; Kfir et al., 2002; Melaku et al., 2006; Mutyambi et al., 2014), and recently in the Mediterranean Basin (Yonow et al., 2017). Owing to its ability to survive in low and high elevations (Kfir, 1993, 1997; Guofa et al., 2001), and on a broad temperature spectrum (Kfir, 1997; Mutamiswa et al., 2017), and higher potential rate of increase and shorter life cycle (Kioko et al., 1995; Kfir, 1997; Ofomata et al., 2000), *C. partellus* gains competitive advantage over other maize stem borers. *Chilo partellus* is described as an invasive species displacing indigenous maize stem borers and becoming the predominant borer pest (Overholt et al., 1994; Kfir, 1997; Ofomata et al., 2000).

Chilo partellus is known to cause severe damage to maize and sorghum wherever it is found (Kfir et al., 2002; Arabjafari & Jalali, 2007). Not only does it damage the vegetative parts of the plant, but it also damages the reproductive parts, causing losses between 24 and 75% (Kumar, 2002), and 80 and 100% in severe infestation in Asia and Africa (Overholt et al., 2000; Arabjafari & Jalali, 2007). The normal damage patterns characteristic of maize stem borers includes bored holes on stems and destroyed internal stem tissue resulting into tunnels (Slabbert & Van den Berg, 2009) and a mixture of rotten, pungent insect and plant tissue debris within the stems. Consequently, the damaged plant is prone to toppling and lodging from any form of disturbance such as heavy rains, storms and strong winds. In addition, *C. partellus* like many other stem borers feed and destroy the growing apex of the maize plant resulting into a condition called deadheart; the growing central leaves die, the plant become stunted and/or dies (Rauf et al., 2017).

Several control and management methods have been employed in an attempt to reduce the damage caused by *C. partellus*. Murenga et al. (2011) presented some control strategies for *C. partellus* and discussed their shortcomings. Resistant maize cultivars have been tried with some success (Ajala et al., 1995; Ahmed et al., 2003; Murenga et al., 2011). Parasitoids such as *Cotesia* spp. (Hymenoptera: Braconidae) and *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) have also been used for control of *C. partellus* with relative success (Kfir et al., 2002; Ahmed et al., 2003). Recently, push-pull technology has gained prominence with a huge adoptability rate especially in the eastern parts of Africa. With regards to push-pull technology, brachiara and Napier grass are among the most commonly used grasses (Van den Berg, 2006; Cheruiyot et al., 2018). The use of pheromones (Beevor et al., 1990), essential oils (Sing et al., 2009) and microorganism-based products (Odindo, 1990; Poinar & Polaszek, 1998) are also gaining grounds. Intercropping is also successfully used for reduction of infestation (Ampong-Nyarko et al., 1994; Pats et al., 1997) up to 30% in maize/sorghum/cowpea-intercropping systems. Other cultural practices such as manipulating planting dates (Van Hamburg, 1979), management of crop residue (Pats et al., 1997), and fertilizer application are also being investigated and used (Van den Berg et al., 1991). However, insecticides applied in foliar and granular formulations are also widely used as preferred solution by farmers since they remain the most convenient control method (Rauf et al., 2017). The insecticides are most effective if applied in the initial stages of an infestation in order to prevent egg hatch and eliminate first and second instar larvae before burrowing in the maize stems (Kfir et al., 2002; Kumar, 2002). In Pakistan, foliar application of fenvalerate, endosulfan, cypermethrin, monocrotophos, quinalphos, and granular application of chlorpyrifos-ethyl and carbofuran are commonly recommended for management of maize stem borers (Mathur & Satyadev, 1992; Katole & Mundiwate, 1995; Bhat & Baba, 2007). Entomopathogens (Odindo, 1991; Gardeze et al., 1998) and novel insecticides such as novaluron, spinosad, emamectin-benzoate have also shown promising results against *C. partellus* (Rameash et al., 2012). Cypermethrin, carbofuran and methamidophos are commercially available in India against the maize stem borers (Khan & Amjad, 2000). According to Van den Berg & Van den Westhuizen (1995), endosulfan and deltamethrin are also used against *Chilo partellus* in South Africa.

Recently, the spotted stem borer was recorded in the Mediterranean Region of Turkey in 2014 (Sertkaya et al., 2014) causing a stir among maize farmers, who have been battling with the voracious indigenous lepidopteran maize stem borer pests such as *Sesamia nonagrioides* (Lefebvre, 1827) (Lepidoptera: Noctuidae) *Ostrinia nubilalis* Hübner, 1796 (Lepidoptera: Crambidae), and *Spodoptera* sp. (Lepidoptera: Noctuidae) especially in the second maize growing season, July-October (Okyar & Kornoşor, 1997). Farmers in Turkey rely almost entirely on insecticides to control maize stem borers. While research is ongoing on the population dynamics and other aspects of *C. partellus*, preliminary results reveal that it is present in maize in Turkey in both the first (April-June) and second (July-October) maize growing seasons, with an infestation rate of 5 to 55% and 20 to 90%, respectively (unpublished data). There are currently no prescribed insecticides in the Turkish market registered for *C. partellus* control. This study was designed to evaluate the potential of some insecticides used against other stem borers for control of *C. partellus*. As such, eight commonly used insecticides in maize production systems used against stem borers in Turkey were screened on eggs and first instar larvae of the spotted stem borers under laboratory conditions.

Materials and Methods

Stem borer colony

The stem borer colony used in this study was the F1 generations of field-collected larvae of *C. partellus*. In June 2018, larvae were collected from a maize field (not sprayed with insecticides) in the Research and Implementation Area of Çukurova University (39°01'50.5" N; 35°21'06.7" E). The larvae were reared on insecticide-free maize stalks (Pioneer Hybrid 1/2013) in the entomology laboratory of Plant Protection Department, Faculty of Agriculture, University of Çukurova. The maize stalks were cut (10 cm) and packed in plastic cups (10 x 10 x 10 cm) covered with a muslin and fitted with a rubber band. The maize stalks were replaced every 5-6 d until pupation. Upon pupation, the pupae were kept in new plastic jars (10 x 10 x 10 cm) lined with a Whatman filter paper for oviposition and covered with a muslin. A cotton ball soaked in water was added in the plastic jar for the adults. Egg masses were laid on the filter paper and these were collected daily for bioassays. The eggs and larvae from these F1 generations were used in the study.

Insecticides used in the bioassay

Table 1 shows the list of selected insecticides commonly used in maize production systems or registered for other lepidopteran stem borers in Turkey. The insecticides which have different mode of actions such as nerve action, chloride channel activator and insect growth regulators were used. Pesticides were used at the manufacturer's recommendation for other lepidopteran stem borer pests of maize, diluted in distilled water. Topical and leaf-dip bioassay were used for the hatching and mortality studies, respectively.

Bioassay

Effect of insecticides on egg hatch

A drop (5µl) of insecticide was applied with a micropipette to egg masses (~40 eggs) when these were at the black-head stage (4-5 d old), after which the eggs usually hatch within 24 h. The egg mass was placed on a maize leaf disk (5 cm), and then placed on a water-soaked cotton in a cup (5 cm diam. x 2.5 cm high). A hole (1 cm), covered with muslin, was perforated on the lid of the cup. There were three replicates per treatment. The setup was kept in a rearing chamber (27±2ºC, 70% RH and 14:10 h L:D photoperiod). The number of eggs hatched was counted 24 h after insecticide application.

Table 1. Tested Insecticides; description, formulations and uses

Active ingredient (ai) / Trade name / Firm name / Country	Con. / Form.	Field rate (ai%)	Rates (ppm)	Chemical family	Mode of action	Crops	Target
Spinosad / LaserTM / Dow AgroSciences / United Kingdom	480 g L^{-1}/ SC	200 ml ha^{-1}	200	Spinosyn	Ingestion and contact. Nicotinic acetylcholine (nAChR) receptor agonist. Nerve action.	Tomato, eggplant, pepper, potato, strawberry, legumes, pome fruits, vegetables, ornamental plants, grass	Lepidoptera, Coleoptera, Diptera
Deltamethrin / Decis® / Bayer CropScience / France	25 g L^{-1}/ EC	500 ml ha^{-1}	5000	Pyrethroid	Ingestion and contact. Paralyses nervous system; knockdown effect.	Pear, vegetables, grapes, pistachio, lentil, chickpea, maize, beet, cereals, sunflower, hazelnut	Mites, thrips, aphids, Lepidoptera, Coleoptera
Lambda-cyhalothrin / Karate® / Syngenta Chemicals / Belgium	50 g L^{-1}/ CS	300 ml ha^{-1}	3000	Pyrethroid	Ingestion and contact. Paralyses nervous system; knockdown effect.	Maize, cotton, apple, grapes, potatoes, beet, tomatoes, nuts, cabbage, cereals, olive	Lepidoptera, mites, aphids
Chlorantraniliprole / Coragen® / DuPont / France	200 g L^{-1}/ SC	150 ml ha^{-1}	1500	Anthanilic diamide	Ingestion. Ryanodine receptor modulator.	Tomato, eggplant, pepper, cucurbit crops, leafy vegetables, maize, peach, quince, plum	Lepidoptera
Indoxacarb / Tunchii® / Astranova / Turkey	150 g L^{-1}/ SC	300 ml ha^{-1}	3000	Oxadiazine	Ingestion and contact. Voltage-dependent sodium channel blocker.	Tomato (field and greenhouse), hazelnut, maize	Lepidoptera
Chlorpyrifos-ethyl / Dursban®4 / Dow AgroSciences / United Kingdom	480 g L^{-1}/ EC	1800 ml ha^{-1}	1800	Organophosphate	Ingestion and contact. Inhibits cholinesterase. Nerve action.	Pear, vegetables, grapes, pistachio, lentil, chickpea, maize, beet, cereals, sunflower, hazelnut, cotton	Lepidoptera
Emamectin-benzoate / Pancart® / Platin Kimya / Turkey	5%/ SG	300 g ha^{-1}	3000	Avermectin	Ingestion. Chloride channel activator.	Pepper, tomato (field and greenhouse)	Lepidoptera
Novaluron / RIMON SUPRA® / Adama / Israel	100 g L^{-1}/ SC	400 g ha^{-1}	4000	Benzoylphenyl urea	Ingestion and contact. Insect Growth Regulator-inhibits chitin synthesis.	Tomato (field and greenhouse), pepper (greenhouse), cotton, soya, cucumber	Lepidoptera

Con.: concentration; Form.: formulation; SC: suspension concentrate; SG: soluble granules; EC: emulsifiable concentrate; CS: capsule suspension.

Effect of insecticides on survival of emerging larvae

The first instar larvae hatched from insecticide-treated eggs were kept separately on fresh insecticide-free maize leaf disks. The leaf disks were kept on moist cotton in plastic cups (5 cm diam. x 2.5 cm high), and then covered with a perforated lid, sealed with a muslin for ventilation. There were three replicates per treatment. The setup was kept in the temperature chamber (27±2ºC, 70% RH and 14:10 h L:D photoperiod). Mortality of the emerging larvae was assessed after 48 h.

Effect of insecticides on first instar larvae

Leaf-dip bioassay was used to evaluate the effect of insecticides on first instar larvae of *C. partellu*s. Fresh maize leaf disks (2 cm) were immersed in insecticide solution for 5 s, and then allowed to dry for 1 h under laboratory conditions (25±2ºC). The leaf disks were then placed on moist cotton in cups and covered with a perforated lid, sealed with a muslin for ventilation. Ten first instar larvae of *C. partellus*, collected from F1 generation of laboratory reared field-collected larvae were placed on the leaf disk with the help of a fine camel hair brush. There were three replicates per treatment. The setup was kept in a climatic chamber set to 27±2ºC, 70% RH and 14:10 h L:D photoperiod. Mortality of the larvae was assessed every 12 h for 3 d (72 h). A larva was considered dead if they did not move after being touched by a fine camel hair brush.

Data analysis

Normality and homogeneity of variance tests for the data were done using Kolmogorov-Smirnov and Levene's test, respectively. Percentage hatch of black-head stage eggs after 24 h was subjected to one-way analysis of variance (ANOVA) and means were separated by post hoc Duncan's multiple range test (DMRT) procedure at a significance level of 0.05. Mortality of emerged larvae was assessed 48 h after hatching. Mortality of hatched larvae after 48 h and first instar larval mortality on insecticide-treated leaves after 72 h was analyzed (one-way ANOVA, DMRT, P<0.05). The mortality was also assessed every 12 h for survival analysis.

Survival analysis, which is generally a set of methods for analyzing data where the outcome variable is the time until the occurrence of an event of interest (mortality), was conducted. Kaplan-Meier survival curves were used to estimate median survival times (LT_{50}; median survival time is the time at which 50% of the first instar larvae survive) 72 h after exposure to the insecticides and their respective 95% confidence intervals (CI) were determined. Hazard ratios after 72 h were estimated using Cox regression to estimate the probability of mortality of first instar larvae occurring in the insecticide-treated leaves compared to the water-treated (control) leaves at any given time. All analyses were done using Statistical Package for Social Sciences (Ver. 23, 2015).

Results and Discussion

Effect of insecticides on hatch of black-head stage egg masses

The effect of various insecticides on hatch of black-head stage egg masses of *Chilo partellus* are shown in Table 2. There was a significant difference (F=41.2, df=8,18, P<0.05) in the mean percentage of hatched egg mass exposed to different insecticides. The smallest percentage hatch (30.6%) was with deltamethrin, followed by t chlorpyrifos-ethyl (86.3%) and lambda-cyhalothrin (89.9%). The percentage of eggs that hatched from the other insecticides was greater than 90%. It is known that the chorion layer of lepidopteran egg is not particularly permeable to ovicidal and toxic chemicals; nevertheless, some chemicals can pass through. In such events, these toxic chemicals can negatively affect embryonic development and/or result in death (Trisyono et al., 2000; Galvan et al., 2005). Of all the insecticides, deltamethrin significantly prevented hatch of the black-head stage egg masses (Table 2). On average, hatch was about three times lower in deltamethrin relative to the other insecticides. It is not clear why there

was inconsistency in the percentage hatch between deltamethrin and lambda-cyhalothrin as they are members of the same chemical class.

Pineda et al. (2004) reported that spinosad diluted in water did not exhibit any ovicidal activity in *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) eggs. However, when diluted in acetone, the percentage hatch was significantly reduced from 80-89% to 36.0% in water and acetone solvents, respectively. The effect of insecticide on hatching and other biological events in insects is affected by the solvent used. According to Adan et al. (1996) the penetration and deposition of insecticide into insect cuticle is facilitated by organic solvents. In another study, the number of *O. nubilalis* eggs hatching after treatment with indoxacarb, novaluron, spinosad and water (control) at the black-head stage was not significantly different (Boiteau & Noronha, 2007). However, when the insecticides were sprayed 2 d before the black-head stage, the number of eggs that hatched was significantly reduced compared to application at the black-head stage by 60, 80 and 8% for indoxacarb, novaluron and spinosad, respectively. Mahmoudvan et al. (2014) also reported that indoxacarb SC (300 mg L^{-1}) and spinosad (480 mg L^{-1}) caused ovicidal control of 86 and 100%, respectively on the egg masses of *Plutella xylostella* (L.,1758) (Lepidoptera: Plutellidae). Hexaflumuron (200 mg L^{-1}), and lufenuron (1000 mg L^{-1}) which are in the same group as novaluron, also gave ovicidal control of 100 and 50%, respectively, in the same study. However, it should be noted that in the experiments of Mahmoudvan et al. (2014), the egg masses used were no more than 10 h old. This suggests that the effect of insecticides on egg hatch also depends on the age of the eggs; the younger the eggs, the more effective the insecticide. High ovicidal effect of novaluron on other lepidopteran has been reported in other studies (Assal et al., 1983; Chockalingam & Noorjahan, 1984).

At the commercial recommended application rate, the percentage hatch of lepidopteran egg masses can be strongly influenced by the solvent (medium) of the insecticide and the age of egg mass. De Smedt et al. (2015) indicated that hydrophobic compounds are more likely to absorb the intermediate and nonpolar poly (*p*-phenylene) PPPs, and therefore can easily attach to the hydrophobic egg surface, causing desiccation of the eggs. For this reason, perhaps, a decreased hatching percentage might have been observed had a hydrophobic solvent like acetone and young egg masses (hours old) been used.

Table 2. Mean percentage hatched black-head stage eggs of *Chilo partellus* 24 h after application of insecticides

Treatment	Replicates	Egg/mass	Mean (%)±SEM	95% confidence interval (CI)
Control	3	35-40	98.2±0.65 c	97.3-99.2
Spinosad	3	30-34	91.6±2.07 bc	88.2-95.0
Deltamethrin	3	32-40	30.6±5.76 a	21.7-39.6
Lambda-cyhalothrin	3	30-40	89.9±1.20 bc	88.0-91.9
Emamectin-benzoate	3	30-36	95.8±0.88 bc	94.5-97.1
Indoxacarb	3	30-32	99.0±0.50 c	97.9-99.0
Chlorpyrifos-ethyl	3	30-37	86.3±1.90 b	83.4-89.3
Chlorantraniliprole	3	32-40	95.1±1.85 bc	92.2-97.9
Novaluron	3	30-40	97.8±1.02 c	95.6-97.8

Number of eggs refers to the number of eggs per egg mass on which topical application of insecticide was made. Sem: standard error of means. 95% confidence interval of percentage hatched eggs of means. The SEM (standard error of means) and the 95% CI was Bootstrapped 1000 times using the bias corrected acceleration. Means in the same column followed by the same letter are not statistically significantly different by DMRT at p<0.05. Each replicate had 10 insects.

Mortality of hatched larvae 48 h after hatching

The mortality rate of larvae 48 h post hatching was also compared across the different insecticides (Figure 1). The percentage mortality was significantly different (F=34.3, df=8,18, P<0.05) across the various insecticides. The highest percentage mortality was observed from emamectin-benzoate (84.5%), and followed by chlorpyrifos-ethyl (84.4%). The lowest mortality was recorded from indoxacarb (23.2%), followed by novaluron (30.5%) and lambda-cyhalothrin (38.2%). The survival of larvae hatched from the insecticide-treated egg masses was impacted by all insecticides. Emamectin-benzoate, chlorpyrifos-ethyl, deltamethrin, spinosad and chlorantraniliprole had higher larvicidal percentages. The dead larvae were probably contaminated by these insecticides as they chew their way out of the egg masses through their mouth or by contact with contaminated surfaces. Indoxacarb is reported to have ovi-larvicidal properties against some Lepidoptera pest such as codling moth and *O. nubilalis* (Boiteau & Noronha, 2007), however, this was not the case in the current study as this chemical had lowest number of dead neonate larvae (Figure 1).
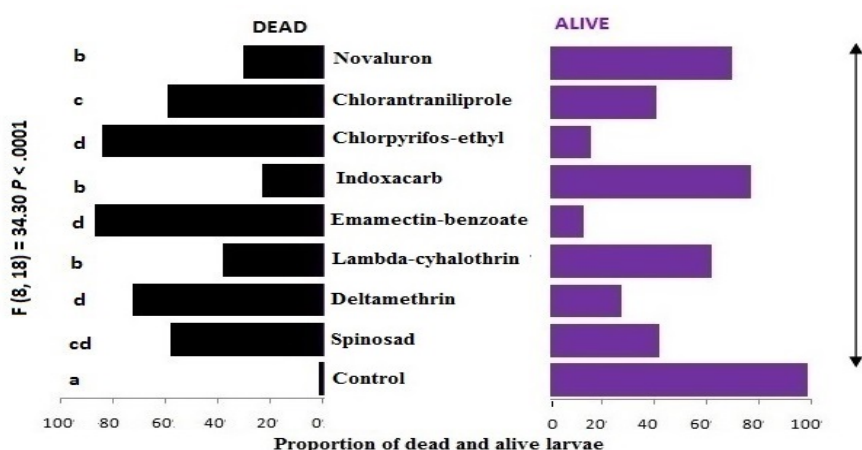

Figure 1. Mean percentage mortality of larvae that hatched from insecticide-treated eggs after 48 h: comparing percentages of dead and alive across different treatments. Bars with the same letter are not statistically different (DMRT, α=0.05). Arrow shows direction of mean comparison.

Effect of insecticides on first instar larvae

The effect of the various insecticides on the first instar larvae 72 h post application is given in Figure 2. The insecticides significantly influenced (F=8.26, df=8,18, P<0.05) the percentage mortality of first instar larvae. The highest percentage mortality was with emamectin-benzoate (96.7%), and lambda-cyhalothrin (96.3%). The smallest percentage mortality was with indoxacarb (62.2%) and chlorpyrifos-ethyl (62.6%). The percentage mortalities recorded in this study were greater than 60% for all insecticides. The effect of insecticides on the first instar larvae revealed that the first instar larvae were very susceptible to many insecticides. Emamectin-benzoate, pyrethroids and spinosad are known to adversely affect larval stages of lepidopterans such as *O. nubilalis, S. nonagrioides, Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae)*, P. xylostella* (Pineda et al., 2004; Boiteau & Noronha 2007; Kurt & Kayis, 2014; Mahmoudvan et al., 2014) including *C. partellus* (Tanwar et al., 2017; Kumar & Alam, 2017). Intoxication of the larvae possibly came from contamination from the leaves as they moved around and during feeding.
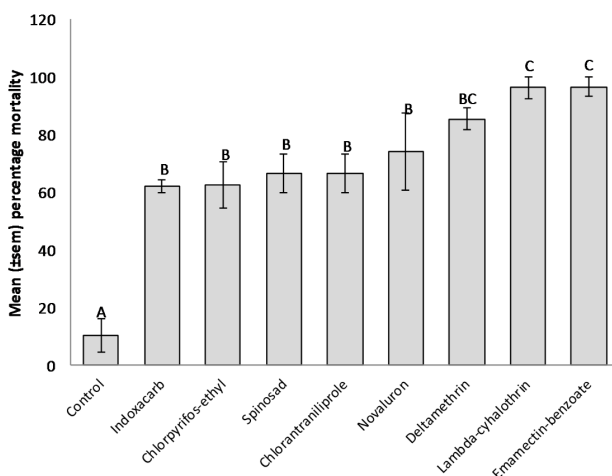
Figure 2. Mean percentage mortality of first instar larvae on insecticide-treated eggs 72 h post exposure to insecticide-infested leaf disks (percentage mortality was transformed using arcsine transformation before analysis). Bars with the same letter are not statistically different (DMRT, α=0.05).

Feeding behavior

On completion of the bioassays (72 h), the number of feeding scars made by the larvae on the pesticide-treated leaves were counted and scored. The values were subjected to Kruskal-Wallis ANOVA. The amount of leaves consumed; indicated by the mean rank was significantly different (χ^2=18.8, df=8, P<0.05). The highest mean rank was obtained with the control and novaluron treatments. The smallest mean ranks were recorded with deltamethrin, lambda-cyhalothrin, emamectin-benzoate and chlorantraniliprole (Figure 3). The feeding capability experiment revealed that these insecticides reduced the feeding activity of first instar larvae, thereby ensuring adequate photosynthesis for the plants. In this study, deltamethrin, lambda-cyhalothrin, emamectin-benzoate exerted the greatest negative effect on the feeding capability of the first instar larvae. In a related study, Hannig et al. (2009) investigated the effect of chlorantraniliprole and seven other commercial insecticides on the feeding behavior of four lepidopteran species, *P. xylostella*, *Trichoplusia ni* (Hübner, 1803) (Lepidoptera: Noctuidae), *S. exigua* and *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae). For time to feeding cessation and reduction in feeding, chlorantraniliprole was the fastest-acting insecticide followed of emamectin-benzoate, indoxacarb, lambda-cyhalothrin, esfenvalerate and methomyl.
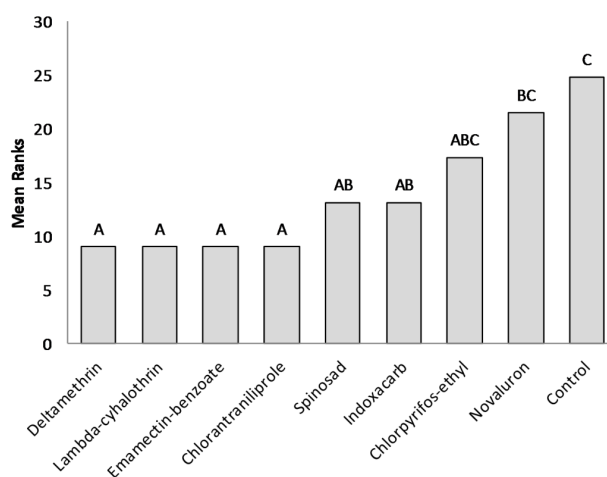

Figure 3. Insect feeding behavior. Mean ranks with the same letter are not significantly different (Duncan's test, α=0.05).

Survival analysis

The mortality of the first instar larvae was assessed every 12 h. Figure 4 shows the Kaplan-Meier curve and Table 3 shows the median time (LT50) and the hazard ratios with their corresponding 95% CI for each insecticide.

The steepness of the slope started from 48 h for deltamethrin, with 4 larvae surviving to the end of the study. The steepness for lambda-cyhalothrin is similar to that of deltamethrin; however, only two larvae survived to the end of the study. The steepness began from 24 h for emamectin-benzoate with one larva surviving to the end of the study. The steepness of the slope for indoxacarb started in the 12 h with 11 larvae surviving to the end. The steepness of the slopes for chlorpyrifos-ethyl and chlorantraniliprole started from 36 h with 11 and 10 larvae surviving to the end, respectively. Novaluron also had a steep slope beginning from 36 h with seven larvae surviving to the end (Figure 4). Survival analysis is widely used in scientific research to evaluate the rate of a certain outcome (mortality) over time. The study revealed that emamectin-benzoate had the lowest median time and the highest hazard ratio.
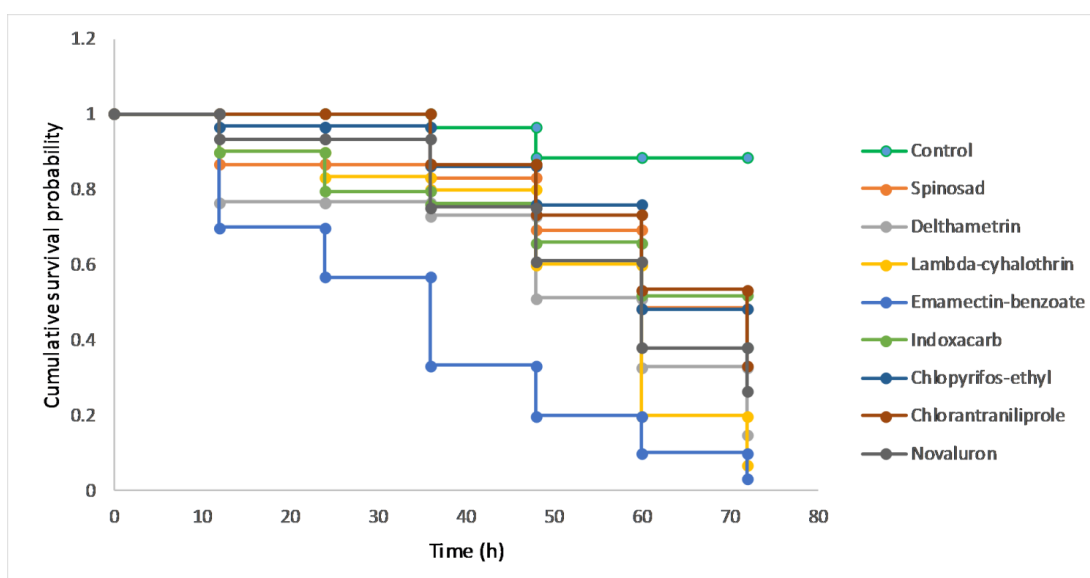

Figure 4. Kaplan-Meier survival curves for first instar larvae of *Chilo partellus* exposed to different insecticide-treated leaf disks after 72 h.

The reference treatment in this study was the control. Since the mortality in the reference treatment never reached 50% in any replicate, it is impossible to calculate median time values for the control. The media time ranged from 36 to 72 h. The shortest median time was recorded with emamectin-benzoate (36h, 95% CI: 27.3-44.7). The highest median time was recorded with indoxacarb (72 h, 95% CI: 56.7-87.3) and chlorantraniliprole (72 h, 95% CI: 61.9-82.1). The median time for all other insecticide was 60 h. For spinosad, the mortality slope was gentle, with 11 larvae surviving to the end of the study. The hazard ratios in this study ranged from 4.91 (95% CI: 1.66-14.6, P<0.0001) with chlorpyrifos-ethyl to 15.6 (95% CI: 5.33-45.6), P<0.0001) with emamectin-benzoate. The mortality of first instar larvae was about 16, 10 and 9 times that of the control with emamectin-benzoate, lambda-cyhalothrin and deltamethrin, respectively.

This study was conducted as an initial attempt to evaluate the effectiveness of insecticides used in maize production system in Turkey in order to develop baseline data against *C. partellus*, a newly recorded pest of maize in Turkey. The findings of this study present the short-term efficacy (acute toxicity) of these insecticides. A better understanding of these insecticides requires a long-term (indirect and subtler effect) study on the physiology and behavior of the target pests and their natural enemies (Biondi et al., 2013; Guedes et al., 2016).

Table 3. Hazard ratios and median time ($LT_{50}s$)

Treatment	Hazard Ratio	95% CI	LT_{50} (h)	95% CI	*P* values
Spinosad	5.13	1.73-15.18	60.0	47.15-72.8	.003
Deltamethrin	8.82	3.04-25.62	60.0	49.56-70.44	.0001
Lambda-cyhalothrim	10.17	3.50-29.52	60.0	55.05-64.95	.0001
Emamectin-benzoate	15.58	5.33-48.56	36.0	27.33-44.68	.0001
Indoxacarb	5.38	1.82-15.93	72.0	56.67-87.33	.002
Chlorpyrifos-ethyl	4.92	1.66-14.58	60.0	50.09 -69.91	.004
Chlorantraniliprole	5.39	1.82-15.80	60.0	50.38-69.62	.002
Novaluron	6.70	2.28-19.72	60.0	56.32-63.68	.001

Omnibus test for the hazard ratios (chi square χ^2=65.6, df=8, P<0.0001), log rank for LT_{50} (chi square χ^2=80.6, df=8, P<0.0001). CI: confidence interval.

Additional experiments are therefore recommended to determine the ecological effects of these insecticides in field trials. Points of focus could be on the rate and time of application. There are numerous advantages of using insecticides with ovicidal and larvicidal activity, targeting eggs, newly hatched larvae and old larva than insecticides that are only larvicidal. Thus, the role of novaluron and chlorantraniliprole should be considered alongside emamectin-benzoate, lambda-cyhalothrin and deltamethrin as part of an integrated pest management plan.

Acknowledgements

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