Efficacy of mating disruption technique against Codling moth [Cydia pomonella L. (Lepidoptera: Tortricidae)] in walnut orchards in Kahramanmaraş

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Abstract
This study was conducted in two walnut orchards including Şebin, Yalova-4 and Sütöymez-2 with Şebin and Sütöymez-2 varieties in 2012 and 2013 in Central district of Kahramanmaraş province. The efficacy of the mating disruption technique was investigated by using dispensers loaded with “(E,E)-8,10-dodecadienol, n-dodecanol,n-tetradecanol 50%, (Z)-11-tetradecenyl acetate 50%” pheromone for the management of Codling moth [Cydia pomonella L. (Lep.: Tortricidae)]. Trial was established as large parcel experimental design having two characters -mating disruption (MD) and control (NP, no pesticide) application. The population dynamics of the pest was monitored by sex attractant pheromone traps. The pheromone dispensers were applied at 1000 dispensers ha⁻¹ (5 dispensers tree⁻¹) and total 3000 dispensers were applied in the orchard. The dispensers were tied to different directions and to a suitable branch located at 1/3 top part of the tree. The emission amounts of dispensers were determined by weekly measuring of the weight of 10 dispensers, and the emission amount was 61 mg h⁻¹ dispenser⁻¹ in MD plot. Counts for fruit damage assessment was evaluated by inspecting 1000 fruits at harvest period. The average infestation rates were calculated as 5.1-21.3% and 4.8-20.1% in MD and NP plot, respectively. As a result of this study, it was determined that the usage of 1000 dispensers ha⁻¹ in walnut orchards can be used effectively as an alternative method instead of chemical control against C. pomonella for organic production and for integrated pest management.

Keywords: Walnut; Codling moth; Cydia pomonella L.; Mating disruption technique

Kahramanmaraş ili cevizlerinde zararlı Elma içkurdu [Cydia pomonella L. (Lepidoptera: Tortricidae)]'na karşı çiftleşmeyi engelleyecek tekniğinin etkinliği

Öz
Bu çalışma; Kahramanmaraş ili Merkez İlçe’de Şebin, Yalova-4, Sütöymez-2 ve Şebin çeşitlerinden oluşan iki farklı ceviz bahçesinde 2012-2013 yıllarında yürütülmüştür. Çalışmada, Isonet-C "(E,E)-8,10-dodecadienol, n-dodecanol,n-tetradecanol % 50, (Z)-11-tetradecenyl acetate % 50" feromon yayıcısının Elma içkıdu [Cydia pomonella L. (Lep.: Tortricidae)]'na karşı çiftleşmeyi engelleyecek tekniğinin etkinliği belirlenmiştir. Deneme; geniş parsellere karşılık 5 ha dozunda, organik tarım ve entegre mücadele üretim çalışmalarında kimyasal mücadeleye alternatif bir yöntem olarak başarı şartı ve entegre mücadelede sistemlerdeki kimyasal mücadelede alternatif bir yöntem olarak başarı şartı şartı ve entegre mücadelede sistemlerdeki kimyasal mücadelede altı büyük governance ile kullanılabileceği belirlenmiştir.

Anahtar Kelimeler: Ceviz; Elma içkıdu; Cydia pomonella L.; Çiftleşmeyi engelleyecek tekniği

1. Introduction
Anatolia is the germplasm center of walnut (Juglans regia L.) as well as many other fruit varieties. Having rich mineral and vitamine sources, walnut is indispensable as a food. It is also a rich income source. Walnut can be cultivated all over the country. World shelled walnut production is 3.5 million tons in 2017. Turkey ranks fourth in the world with...
215,000 tons (TUİK, 2018) of walnut production, ranking after China, USA and Iran, respectively. According to regions, Mediterranean and Middle Anatolia regions are at the first rank with 15% of the total production in Turkey. Western Black Sea and Aegean regions follow with 14% production (Yavuz, 2012).

There are numbers of pest and diseases causing phytosanitary problems and crop loss in walnut plantations (Güçlü et al., 1995; Çevik, 1996; Gökþürk, 2001; Ginzel, 2010; Canlıhoş et al., 2014; Anonymous, 2017). Codling moth [Cydia pomonella L. (Lepidoptera: Tortricidae)] is one of the insect pests among them. This pest cause damage in apple, pear and quince as well as walnut and it is a key and economic pest for these fruits. The pest dispersed to the whole world except Japan and West Australia. Codling moth has 1 to 3 generations per year, varying from region to region (Canlıhoş, et al., 2014; Anonymous, 2017).

Numbers of studies were conducted on the biology, damage and management of Codling moth damaging apple and pear in Turkey and in the world until now but there are fewer studies on walnut (Cisneros and Barnes, 1974; Dindar, 1995; Dindar and Ecevit, 1996; Shorey and Gerber, 1996; Light et al., 2005; Anonymous, 2011; Zeki and Özdem, 2013; Canlıhoş et al., 2014; Demir and Kovancı, 2015; Anonymous, 2017). Larvae of Codling moth directly feeds on walnut fruit. The pest feed both inside and peel of the fruit. It causes yield loss because of nutlet drop and also reduces quality and market value of the crop. First instar larvae feed inside of the fruit. Second and third instar larvae generally feed on green peel of the fruit. Crop loss up to 20-50% may occur if no management strategy is applied (Zeki and Özdem, 2013; Canlıhoş et al., 2014; Anonymous, 2017). That kind of damage is undesirable for both growers and consumers.

Monitoring the adult population development and the management of Codling moth is conducted by sex pheromone traps and dispensers at the present time (Witzgall et al., 2008). Mating disruption is among these methods which is rather effective. In a study conducted in walnut orchard in California (USA), it is notified that Isomate-C dispensers were used in 1000 dispensers per ha and as a result C. pomonella adult numbers were decreased 90% which shows mating disruption of the pest was an effective method for the management of the pest (Light et al., 2005). In another study conducted in walnut orchards in Bursa, Turkey in 2012-2013 reported that they used C. pomonella dispensers as 1000 dispensers/ha dose and found out the average damage on fruit as 1.3% at the assessment during fruit harvest (Demir and Kovancı, 2015).

Over the past 50-60 years, agricultural techniques based on chemical application has been given importance in order to reduce the losses originating from plant health problems to a minimum level and to provide the nutrient needs of increasing human population in the world. But, these techniques brought some problems like high costs, the destroying of natural balance, environmental pollution, resistance and residue. In addition to these, clean nutrient demand of consumers necessitates the growers to give priority to alternative methods.

This study was conducted in two walnut orchards including Şebin, Yalova-4, Sütýemez-2 and Şebin varieties for the aim of determining the efficacy of mating disruption method as an alternative method to chemical application for the management of Codling moth (C. pomonella)-key pest of walnut orchards in Central district of Kahramanmaraş province in 2012-2013. According to the data obtained from this study, it is determined that mating disruption method will be useful for control of C. pomonella during organic production and for integrated pest management.

2. Materials and Methods

In the study of mating disruption technique for Codling moth [Cydia pomonella L. (Lepidoptera: Tortricidae)], Isonet-C (Isomate C Plus®, Sumitomo Corporation, Turkey) pheromone dispensers [(E, E)-8,10-dodecadienol, n-dodecanol, n-tetradecanol 50%, (Z)-11-tetradecenyl acetate 50%] were used (Anonymous, 1999). Population dynamics of this pest was monitored by delta pheromone trap (Treeco, pherocon CAP, Ca, USA).

Trial design was large parcel experimental design having two characters; (Mating...
disruption (MD), Control (NP) no pesticide). Meteorological data such as air temperature, relative humidity (%), and wind velocity was obtained from I-Metos meteorology station (Pessl Instruments GmbH; Weiz; Austria) placed in the trial orchard. Studies were conducted in a 15 years old orchard located in Dereköy (Dulkadiroğlu district) country of Kahramanmaraş province of Turkey in Sütcü Imam University Agriculture Faculty Nuts Research and Application Center (Altitude: 930 m, 37°35'27" North and 37°03'28" East) in 2012-2013. The orchard was covering with 3 ha including 573 trees and was established with cv. ‘Şebin’, ‘Yalova-4’ and ‘Sütyemez-2 local Walnut varieties. The control plot was 0.5 ha including 96 trees and 550 m away from trial orchard. Şebin and Sütyemez-2 variety trees were 12 years old.

In order to monitor the adult population dynamics of C. pomonella, one sex pheromone trap in the NP orchard and two traps in the MD trial orchard were hanged on 16 April 2012 in the first year and on 08 April 2013 in the second year (Anonymous, 2011; 2018). Traps were hanged 1.5 m height to south direction of trees when the sum of effective temperature was reached to 80 degree-days (DD) starting from the biofix date 1st of January. The traps were checked two times a week until the first adult was caught. After the capture of the first adult, trap was checked weekly until harvest. The number of adults on the trap was counted and recorded in every check date. Isonet-C pheromone dispensers with 1000 dispensers/ha dose (5-6 dispensers tree⁻¹) were loaded to the trial area within 1-3 days after the first adult was captured in traps. The dispensers were tied to the appropriate branches located in the upper 1/3 part of the trees at various directions (Anonymous, 1999; Light et al., 2005; Demir and Kovancı, 2015; Anonymous, 2018). The method applied one time throughout the year. Similar dose of dispensers were tied to the other trees excluding walnuts in the orchard (Femenia-Ferreri et al., 2007; Anonymous, 2011; Demir and Kovancı, 2015).

Assessment of the trial was done by counting the damaged and clean fruits among 1000 randomly selected ones (Anonymous, 2018). The damaged fruit rate in MD trial orchard was compared with 6% threshold while this rate was 2% for NP orchard (Anonymous, 2017; 2018).

For this aim, twenty trees having high yield were selected to conduct damage assessment counts from 4 different directions and from center of each orchard. A few days before harvest, 50 random fruits on each of 20 trees were checked to determine if damaged or clean. According to results of this study, the statistical analysis of fruit infection rates in 2012 and 2013 were calculated with Chi-square test via SPSS 23 Statistic Programme.

In order to determine the pheromone release amounts in Isonet-C dispensers, 10 of them were tied to a 1.5 m rope at intervals of 10 cm and then hanged on a suitable tree in the orchard. These dispensers were weighed weekly on assay balance starting from the beginning of the trial and continued to weigh until the end of the trial. Result was shown on a pheromone release graph. Throughout the trial, no chemical application was done at the MD orchard against walnut diseases and insect pests. As there was not any host plant like apple, pear and quince closer than 500 m to the trial orchard, a buffer zone was not constituted to avoid external infestations.

3. Result and Discussion

Isonet-C pheromone dispensers were tied to the trees with a 1000 dispensers ha⁻¹ dose 2 May 2012 in the first year and 30 April 2013 in the next year as one application throughout each year (Figure 1). In a study conducted in Spain, it was noted that pheromone dispensers of C. pomonella were applied only one time in a year and the dispensers released pheromone 143 days along (Femenia-Ferreri et al., 2007). In both trial years 3000 dispensers were used. While 2850 of them were tied to walnut trees, 150 of them were tied to trees around the trial orchard (Figure 1). A buffer zone against external contamination wasn’t constituted because trial orchard was large enough and it was completely isolated. Moschos et al. (1998) reported that mating disruption technique should be applied at an isolated and large orchard in order to lower the negative effect of mated females flying from adjacent orchards. Infestation rates of C. pomonella in MD and NP orchards in 2012-2013 are shown in Table 1. A thousand fruit samples were checked both in MD and NP orchards to obtain rate of C. pomonella damage.
Figure 1. Isonet-C dispensers (a) and an applied dispenser (b) on a walnut tree

Table 1. Damage rate percentages of *Cydia pomonella* in mating disruption and control orchards in Kahramanmaraş in 2012-2013 (MD: Mating disruption, NP: no pesticide)

| Years | Direction | Amount of fruit sampled | Amount of clean fruit | Amount of damaged fruit | Damage rate (%) |
|-------|-----------|-------------------------|-----------------------|------------------------|-----------------|
|       | MD        | NP                      | MD                    | NP                     | MD              | NP              |
| 2012  | East      | 207                     | 197                   | 10                     | 4.8             | 23.6            |
|       | West      | 210                     | 195                   | 15                     | 7.1             | 25.8            |
|       | North     | 204                     | 195                   | 9                      | 4.4             | 16.1            |
|       | South     | 207                     | 196                   | 11                     | 5.3             | 19.8            |
|       | Center    | 201                     | 193                   | 8                      | 3.9             | 20.8            |
|       | Total     | 1029                    | 980                   | 53                     | 5.1             | 21.3            |
| 2013  | East      | 201                     | 191                   | 10                     | 4.9             | 14.8            |
|       | West      | 203                     | 191                   | 12                     | 5.9             | 24.1            |
|       | North     | 203                     | 194                   | 9                      | 4.4             | 22.4            |
|       | South     | 204                     | 194                   | 10                     | 4.9             | 20.3            |
|       | Center    | 201                     | 193                   | 8                      | 3.9             | 18.5            |
|       | Total     | 1012                    | 963                   | 49                     | 4.8             | 20.1            |

Average damages in MD and NP orchards were 5.1-21.3% and 4.8-20.1% in 2012 and 2013, respectively (Table 1; Figure 2b).

The lowest damage rate was 3.9% in the center of the MD trial orchard while the highest rate was 7.1% in the west part of the orchard at the direction of prevailing wind in the first year. The damage rates were between 3.9-5.9% in the second year (Table 1).

Although dispensers were applied 50 m width towards the prevailing wind in MD orchard, the highest damage rate (7.1-5.9%) was occurred at the west side of the orchard. This was because pheromone cloud above the orchard was dragged along to the inner part of the orchard by the wind blowing from the west. Ogawa (1997) revealed that pheromone release increased 13% but pheromone density at the top of the orchard but it was decreased at 50% when wind speed increased from 1.0 m to 2.5 m per second. Damage rates in MD orchard was lower than 6% -threshold value- in both years.

Acceptable damage threshold of mating disruption method for Grape berry moth (*Lobesia botrana*), Codling moth (*Cydia pomonella*) and Peach twig borer (*Anarsia lineatella*) is also reported as 6% in Turkey (Anonymous, 2018). It was reported that applied 1000 Isomate-C dispensers/ha in a walnut orchard in Bursa and average damage was found as 1.3% in 2012 and 2013 (Demir and Kovanci, 2015). In a study conducted by Light et al. (2005) in California (USA) walnut orchards, number of adult *C. pomonella* on traps decreased to 90% when 1000 Isomate-C dispensers were applied per ha and the researchers reported that mating disruption method was successful against *C. pomonella*. 

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Anonymous, 2018. 
Demir and Kovanci, 2015. 
Light et al. (2005).
As to similar studies abroad, it is notified that MD method is more efficient when applied at least two years consecutively in the same area (Moschos et al., 1998; Kast, 2001). In the same way, damage rate of the second year was found lower than the first year in this study (Table 1). The statistical analysis of damage rates in MD and NP trial orchards are shown in Table 2.

As shown in Table 2, the difference between damage rates of MD and NP trial orchards is found statistically important (5% level) according to Chi-square test. During the two years’ study, sexual attractant traps were hung in MD and NP trial orchards in order to monitor adult *C. pomonella* populations (Figure 1a). Adult flight graphs drawn by using the data obtained from these traps were shown in Figure 3. As shown in Figure 3, the first *C. pomonella* adults were captured on 29\textsuperscript{th} of April in both years on pheromone traps. In a study conducted in Çorum (Oğuzlar), Zeki and Özdem (2013) notified that first *C. pomonella* adults were caught on 21 April, 30 April and 01 May in 2007, 2008 and 2009 respectively. Similarly, it is reported that first *C. pomonella* adults were caught on the first half of May in Bursa walnut orchards in 2012-2013 (Demir and Kovancı, 2015). In a study conducted in California, first *C. pomonella* adults were caught in the beginning of May and flight of first adults lasted until the middle of May. According to this study, next generation adults emerged in June-July and August-September. The off springs mixed with each other and flight of adults ended in the second half of September (Anonymous, 2017). In this study, *C. pomonella* population on traps in MD orchard was lower than the population in NP orchard and also was under damage threshold. This situation showed that mating disruption method against *C. pomonella* was effective in Kahramanmaraş walnut orchards.

![Figure 2. Pheromone trap of *Cydia pomonella* (a) damage on walnut fruit (b)](image)

Table 2. Statistical analysis of *Cydia pomonella* damage rates in mating disruption applied and control orchards in Kahramanmaraş in 2012-2013 (Chi-square, MD: Mating disruption, NP: No pesticide)

| Years | Characters of the trial | Analysis | MD | NP |
|-------|-------------------------|----------|----|----|
|       | Average                 |          | 10.6| 43.8|
|       | Variance                |          | 7.300| 64.700|
| 2012  | Number of sampling      |          | 5   | 5   |
|       | S.D. [D.F.]             |          | 4   | 4   |
|       | Chi-square              |          | 101.309*|     |
|       | Average                 |          | 9.8 | 41  |
|       | Variance                |          | 2.200| 59.000|
| 2013  | Number of sampling      |          | 5   | 5   |
|       | S.D. [D.F.]             |          | 4   | 4   |
|       | Chi-square              |          | 95.811*|    |

* = Significant (Alfa 5% level)
According to the results of various studies conducted in Turkey, 10-15 C. pomonella adult should be caught on pheromone traps weekly in order to decide the management of this pest (Anonymous, 2017; 2018). In this study conducted in Kahramanmaraş, number of adults caught on monitoring traps in 2012 were higher than 2013. Adults were caught between 29th of April and 9th of October in both years. In the first year of the study, 263 adults were caught in NP orchard while only 63 were trapped in MD orchard. Similarly in the second year (2013), totally 238 and 18 adults were trapped in NP and MD trial orchards respectively (Figure 3).

In order to determine the pheromone release rate (mg week⁻¹) of Isonet-C dispensers, each year of this study, 10 dispensers were weighed in the laboratory and then hanged to a tree in the MD trial orchard. The weight of these dispensers were measured weekly until the end of the trial and the release rate graphs were drawn by using this data (Figure 4). As shown in Figure 4, the beginning weight of 10 dispensers was 16.9 g in both years. 190 mg pheromone was loaded in each dispenser. This means that pheromone was 1.9 g and the rest 15 g was tare consisting wire and plastic. The final weight of 10 dispensers were 15.0 g in the days when the pheromone release ended on 04 September 2012 and 11 September 2013. The difference between final weight and tare was zero which shows that the pheromone loaded on 10 dispensers (1.9 g dispenser⁻¹) were released entirely during trial span of 125 and 134 days. Likewise, in a study conducted in California walnut orchard, Isonet-C pheromone dispensers were applied 1000 dispensers per ha and pheromone release span was 120 days (Anonymous, 2011; 2017). As to the study conducted in Kahramanmaraş, dispensers released pheromone for 125 days in 2012 between 2nd of May-4th of September and for 134 days in 2013 between 30 April-11 September (Figure 4).
Figure 4. Pheromone release rates of Isonet-C dispensers in mating disruption trial orchard in 2012-2013

- Pheromone release amount averagely 130 days in both years was 1.9 g.
- Total pheromone release of 1000 dispensers in an hour is calculated as below:
  - $1900 \text{ mg} \times \frac{130 \text{ days}}{1 \text{ year}} = 14.6 \text{ mg dispersion}^{-1} \text{ day}^{-1}
  - $1.46 \text{ mg} \times \frac{24 \text{ hours}}{1 \text{ day}} = 0.060 \text{ mg dispersion}^{-1} \text{ hour}^{-1}
  - $0.060 \text{ mg} \times \frac{1000 \text{ dispensers}}{1 \text{ ha}} = 60 \text{ mg} \text{ 1000 dispensers}^{-1} \text{ ha}^{-1} \text{ hour}^{-1}

Molinari and Cravedi (1992) notified that 20 mg ha$^{-1}$ hour$^{-1}$ release rate was an adequate rate but 70 mg ha$^{-1}$ hour$^{-1}$ pheromone release rate was reported more appropriate for larger and 4-5 m long trees. Similarly, reported that pheromone release rate of dispensers should be 1-2 g ha$^{-1}$ day$^{-1}$ for an effective mating disruption. It is a known fact that crown of the tree and intensive leaf area play an important role for mating disruption method. Temperature and wind speed has a significant effect on pheromone release quality and amount during mating disruption studies (Neumann, 1993).

Depending on descending amount of pheromone, pheromone release rate decreases but this rate is balanced again with the increasing temperature. High wind speed decreases pheromone intensity at the roof of the orchard. High amount of mating is expected in a still weather condition after such high speed wind which is a risk for the effectiveness of the MD method. Millar et al. (1997) emphasized that an ideal dispenser should not be effected by high temperatures, therefore should has a stable release that makes equal pheromone releases in any kind of weather condition.

Climate change graph drawn by using the data of 5 days interval air temperatures, air humidity and wind speed received from Hobo climate data logger in MD orchard is shown in Figure 5. Figure 5 shows that pentat temperature was 16.4°C and air humidity was 71.2% on 29 April 2012 on the day first adults of C. pomonella was trapped in MD orchard. First adults of second year were caught on 29 April 2013. Air temperature and air humidity were 21.5°C and 37.8% respectively.
Development lower threshold and thermal constant of *C. pomonella* was reported as 10.0°C and 538-656 degree-days by Bodenheimer (1958). Similarly, development threshold of the pest is reported as 10.0°C and thermal constant for a single generation was reported as 619.0 degree-days in a study conducted in walnut orchard in California (Pitcairn et al., 1992).

Minimum and maximum temperatures calculated by five days intervals were 15.9-31.4°C and 12.6-27.5°C between 01 May and 22 September respectively while relative humidity values were 21.4-71.2% and 22.4-87.4% in the same period in 2012 and 2013. In a study conducted by Demir and Kovanci (2015) in walnut orchards in Bursa, the fortnightly temperature values were reported to be 16.0-27.0°C during the period when *C. pomonella* adults were active in nature in May-October.

Average wind speed was 2.6 (1.4-5.4) and 0.6 (0.1-2.0) m sec⁻¹ in the same period of both years. As shown in Figure 5, the wind speed was above 2.5 m sec⁻¹ in the first year and below this value in the second year. The low efficacy of MD method calculated for the first year is thought to be the effect of high wind speed and unstable pheromone release caused by wind speed (Figure 4).
The key pest and diseases in MD trial walnut orchard in Kahramanmaras were C. pomonella and walnut anthracnose (or walnut black spot) Gnomonia leptostyla. The other diseases were downy leaf spot (Microstroma juglandis), black pustular dieback of Juglans spp. (Melanconium juglandinum), Root and crown rot (Phytophthora spp.) and Leucostoma cancer (Leucostoma cincta). Other pests were reported as aphids (Callaphis juglandis and Chromaphis juglandicola), walnut blister mites (Eriophyes tristriatus and Aceria erinea), lace bugs (Stephanitis pyri), leopard moth (Zeuzera pyrina), yellow issued planthoppers (Agalmatium flavescens) and Walnut leaf miner (Caloptilia roscipennella) (Canıhoş et al., 2014).

The significant pests and diseases were checked according to Plant Protection Technical Instruction. Cultural precautions were taken in the first place. In both years any plant health problem was confronted except key pests. For this reason, no plant protection application was done that can effect MD technique in the orchard. This was because of well air circulation in the orchard due to the appropriate row spacing, medium tree crown height, a well maintenance and appropriate climate conditions.

4. Conclusion

The efficacy trial for Isonet-C mating disruption dispensers against C. pomonella, the key pest of walnut orchards in Turkey-was done in Kahramanmaras walnut orchard. The method was tested for the first time in Turkey walnut orchards. As a consequence, 1000 dispensers ha⁻¹ was found adequate and efficient. In the study, the damage rate of C. pomonella in the chemical spray applicable control orchard was found high 21.3 and 20.1% in 2012 and 2013 while these values were in acceptable limits as 5.1 and 4.8% and was under 6%, the acceptable damage threshold for MD treatments.

According to these findings, when the residues, natural balance, human and environment health are taken into consideration, mating disruption method is concluded to be used successfully during organic walnut cultivation as an alternative of chemical control in integrated pest management studies.

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