Alternative control agents of the dried fruit mite, *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) on dried apricots

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Received: 19 September 2018 Accepted: 8 November 2018 Available online: 31 July 2019

ABSTRACT: Turkey is the biggest dried apricot producer and exporter of the world. *Carpoglyphus lactis* (L.) (Astigmata: Carpoglyphidae) is the most common species on dried apricots. When C. lactis feeds on the fruit sugar of the dried apricot, the mite accelerates the development of mould fungi and causes unwanted flavours and odours, due to its digestive products, secretions and dead bodies. These contaminants cause skin diseases and some disorders of the digestive system in humans. Fumigants such as methyl bromide and phosphine are used for the control of this harmful species all over the world. However, methyl bromide has been restricted in many countries including Turkey, because the chemical depletes ozone layer and causes acute deaths in humans. Biological studies have shown that reducing the amounts both of oxygen and humidity negatively affects the population development of *C. lactis*. Based on this evidence, the effects of different physical agents, namely ferric oxide and ozone gas (oxygen depleters), and calcium chloride and silica gel (humidity reducers) to packaged dried apricot infested with *C. lactis* were studied. The lethal dose concentrations and lethal times of each product were determined by probit analysis. In this study, the female mites were successful controlled with all of the physical agents. A death rate of the females of 99% was most rapidly obtained with ozone gas treatment, followed by ferric oxide. Ozone gas was particularly toxic, causing a significantly high level of mortality after application at 44.4 mg/L (LT₉₉= 39 hours). When ferric oxide was applied a dose of 9000 mg/L volume and higher doses, it killed all the female mites within 3 days. Above the dose of calcium chloride at 3000 mg /L killed all of the females within 81 hours. Lastly, the silica gel affected mites over a longer period i.e., two weeks and at a higher dosage (56000 mg/L volume).

Keywords: *Carpoglyphus lactis*, control, desiccants, dried apricot, oxygen absorbers, ozone.

INTRODUCTION

The dried fruit mite, *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) is one of the main pest mite of dried fruits (Hughes, 1976; Özer and Toros, 1978). This stored-product pest mite is found generally on food substances containing sugars from which bacterial activity gives rise to lower fatty acids such as lactic, acetic and succinic acids (Hughes, 1976). This mite was the first found in İzmir on dried fruits such as apricots, figs and raisins with 53.3% infestation rate (Özer and Toros, 1978; Genç and Özar, 1986). After this study, *C. lactis* was found as the most abundant mite in 69% of the total mites in stored dried apricots in Malatya, Elazığ and İzmir province of Turkey (Çobanoğlu et al., 2004).

Turkey is one of the world's leading producers and exporter of dried apricot with the first rank (Anonymous, 2017a; 2017b; 2017c). After sun-drying, the apricot fruits could be safe in terms of microbial activity providing intermediate moisture due to below certain levels of water content. The loss in fruit moisture content of dried apricots result in the accumulation of sugars as crystals first on the surface and then within the fruit tissue. Consequently, *C. lactis* feeds by the fruit sugar of the dried apricot and then mold fungi develop on digestive products, secretions, dead bodies of the mite the dried fruits. These

occur unwanted flavour and odours. Infestation of *C. lactis* on dried apricots also causes skin diseases, some disorders in the digestive system and allergy on humans (Hughes, 1976, Özer and Toros, 1978; Genç and Özar, 1986; Özer et al., 1989; Turanlı, 2003; Çobanoğlu et al., 1996; Aksoy et al., 2004; Öztekin et al., 2007; Güldalı and Çobanoğlu, 2010; Hubert et al, 2011; 2015).

Single common control method of C. lactis is fumigation with some chemicals such as methyl bromide and phosphine. In recent years, alternatively some chemicals, such as carbonyl sulphite, sulphuryl fluoride, cyfluthrin and iodomethane are applied in the control of this mite (Ferizli et al., 2004; Meyvacı and Fatih, 2007; Aksoy et al., 2008; Şen et al., 2009). But these compounds cause some problems: (1) Human health: they cause acute deaths in personals, (2) Environmental problem: destruction of ozone layer (methyl bromide), (3) Safety problem: corrosive and inflammable in buildings and transporters (phosphine), (4) Ecological problem: they are broad spectrum pesticides, eradicates non-target organisms, (5) Application problem: fumigation difficulties and slow effects (phosphine), (6) Resistance problem: resistance development in some mite population. Since methyl bromide has got thinning effect of the ozone layer, its use is restricted in many countries implemented the Kyoto Pro-



tocol (Emekçi et al., 2004; Ferizli et al., 2004). MeBr has been gone to be phased out since 2007 according to the Methyl Bromide Action Plan of the Turkish Government (Desmarchelier, 1998, Anonymous, 2017d). For this reason, there is a need for new environmental friendly control methods.

Astigmat stored-product pest mites which rely solely on cutaneous respiration, are thus weakly sclerotized and vulnerable to desiccation (Collins, 2006). Development and/or reproduction of *C. lactis* varies depend on temperature, humidity and oxygen levels. The adult's life span reaches maximum level at 18°C and 65% relative humidity and the most fecundity was observed at 28°C and 80% humidity conditions. C. lactis may die in low humidity and at high temperatures (Cunnington, 1985; Emekçi and Toros, 1989; Bell and Conyers, 2002; Güldalı and Cobanoğlu, 2010). Previous biological studies have shown that reducing amounts of oxygen and/or humidity was negative affected development of C. lactis (Longshu et al., 1992; Ferizli and Emekçi, 2000; Collins, 2006; Sen et al., 2009; Aksov et al., 2012). Based on this evidence, different physical agents could be affected on C. lactis. For example, ozone gas depletes oxygen and is oxidative agent; ferric oxide, calcium chloride and silica gel are humidity reducers and ferric oxide is also oxygen absorbent (Yıldırım et al., 2018). Until now, some previous studies about increasing carbon dioxide or decreasing humidity have given quite successful results (Emekçi et al., 2004; Wang et al., 2008; Işıkber et al., 2015). But, there is no study about the effects of ferric oxide, ozone gas, calcium chloride and silica gel against C. lactis. The aims of this study were to determine the lethal concentrations (LC_{50}) LC₉₀ and LC₉₉) and lethal times (LT₅₀, LT₉₀ and LT₉₉) of these physical agents by probit analysis in laboratory conditions during 2017-2018 years.

MATERIAL AND METHODS

Mite culture

This study performed at Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, Toxicology and Acarology Laboratory in climate chambers during 2017-18. Commercial dried apricots were obtained from a market. The apricot fruits were sterilized with ethyl alcohol (70%). Also, all experimental materials and apricot fruits were dried and sterilized under an UV-chamber. Carpoglyphus lactis population was collected from dried apricots a traditional market of Bursa province (Turkey). The species was identified based on morphological characters with a light microscopy (Hughes, 1976). For mass rearing of mite population on dried apricots, plastic boxes contain dried apricots placed on moistened cotton were used. The mite colony was mass reared in a separate insectarium with a 16-h light (at 27 ± 1 °C and 60 ± 5 % RH) / 8-h dark (23 ± 1°C and 60 ± 5% RH) cycle. Light was provided with white lamps.

Bioassays

Lethal concentration (LC_{50} , LC_{90} , LC_{99}) and lethal time (LT_{50} , LT_{90} , LT_{99}) of ozone gas (O₃), ferric oxide, silica gel

and calcium chloride for *C. lactis* were calculated by probit analysis method using results of modified fumigation bioassay (Simon, 2014). In O₃ lethal concentration bioassays, dried apricot fruits infested with 50 same age female mites were putted in tulle packets placed in 250 cc glass Erlen jars. The jars with valve system were sealed and their airs were vacuumed by a pump. After, the different doses of O_3 (mg/m³) were applied with an Ozone generator. Non-ozonation Erlen jars were used as control. In ferric oxide bioassays, single dose ready ferric oxide packagings (3.5 gr) were used, but these putted in different volume glass jars (100, 250, 500, 1000 etc.). Dried apricot fruits infested with 50 female mites putted in a tulle packet were placed to the jars. Then, the packages were quickly thrown into these jars, because the material absorbs quickly ambient oxygen when vacuumed ferric oxide packagings are opened. The jars without ferric oxide were used as control. Similar method was used for Silica gel (2 gr/package) and calcium chloride (2 gr/package). At least, five or more different doses of each agent were applied. The treatments were carried out three replicates with 50 female mites. Live mites/insects were counted at each 24h. They were considered alive if they that were able to walk at least one body length when probed with a paintbrush while viewed under a stereomicroscope. Mortality data obtained from bioassays were corrected using the Abbott formula (Abbott, 1925). The numbers of alive and dead mites were subjected to probit analysis (SPSS 23. version) to determine LC values for each agents (Simon, 2014). In lethal time bioassay, same methods were used, but LC₉₀ value of each agent was applied at least seven times (hours).

RESULTS

The toxicities of four physically agents namely O_3 , ferric oxide, calcium chloride and silica gel to *C. lactis* females were examined by the modified fumigation method and the probit analysis results are summarized in Table 1. All physically agents killed females of C. lactis in different doses and times. Based on slope value, the O₃ gas response of *C. lactis* females was heterogeneous (the slope of the curve was significantly not steeper) and reflected more variability among individuals. Forty-eight hours after O₃ gas application, its median lethal dose was found as 16.04 mg/L for *C. lactis* females. The highest lethal doses, LC₉₀ and LC₉₉, were calculated as 30.49 and 42.28, respectively. The confidence limits of all this lethal doses were very narrow. In ferric oxide bioassays, sixty hours after application of the agent on dried apricot fruits, the median lethal dose of ferric oxide was 3232 mg/L for C. lactis females. The highest doses, LC90 and LC99, were estimated as 9145 and 21351mg/L, respectively. Different to previous agent response, ferric oxide response of C. lactis females was more homogeneous (b>2). Seventy-two hours after calcium chloride application, the median lethal dose of calcium chloride was found as 1052 mg/L for C. lactis females. The highest doses, LC90 and LC99, were calculated as 3044 and 4670 mg/L, respectively. Similar to O₃ gas response of *C. lactis* females, the mite response was heterogeneous (b<2) but reflected less variability among individuals. In silica gel bioassays, 240 hours after silica gel application, its median dose was 35063 mg/L.

Table 1. The probit analysis and lethal concentration results of O ₃ , ferric oxide, calcium chloride and silica gel for <i>Carpo</i> -
glyphus lactis.

		03	Ferric oxide	Calcium chloride	Silica gel
Number of experimental dose		7	8	12	7
Time (hours)		48	60	72	240
Death rate of control indi- viduals (%)		3.4	6.0	10.23	4.0
Slope±SE		0.089±0.05	2.84±0.173	0.643±0.042	2.105±0.137
LC ₅₀ (mg/L)		16.04	3232	1052	35063
95% confi-	Highest	13.9	1734	341	31828
dental lim- — its I	Lowest	18.1	5311	2055	39406
LC90 (mg/L)		30.5	9145	3044	56407
95% confi-	Highest	27.7	5522	2046	49924
dental lim- — its I	Lowest	34.2	30340	6803	66742
LC99 (mg/L)		42.3	21352	4670	73808
95% confi-	Highest	38.01	10477	3110	64094
dental lim- — its I	Lowest	48.2	170170	11000	89611
X ²		23.7	232.23	487.98	27.154
Probability		0.03	< 0.01	<0.01	0.012

The highest lethal doses, LC_{90} and LC_{99} , were estimated as 56407 and 73808 mg/L, respectively. But, the response of *C. lactis* females to silica gel was more homogeneous (b>2).

(b>2). In action C_{90} values for four physically agents to *C. lactis* females were examined by the modified fumigation method and the probit analysis results are summarized in Table 2. The median lethal times (LT₅₀) of LC₉₀ findition on c values for O₃, ferric oxide, calcium chloride and silica gel were found as 13.43, 48.70, 38.48 and 219.7 hours, respectively. The lethal times (LT₉₀ and LT₉₉) killed 90%

and 99% of the mite population were estimated as 27.54 and 39.04; 62.16 and 73.13; 62.09 and 81.05; 332.3 and 424.2 hours; for O_3 , ferric oxide, calcium chloride and silica gel, respectively.

DISCUSSION

This study is the first report of the control for *C. lactis* females on dried apricot to tested physical agents namely O_3 gas, ferric oxide, calcium chloride and silica gel. The results obtained from this work showed that the females of *C. lactis* can be affected from all of physical agents depending on dose and time. The low dose of O_3 gas killed 90 percent of *C. lactis* females within two days. Until now, there has no attempt about control of *C. lactis* females with O_3 gas. But, our findings are consistent with other studies that O_3 gas reported to be effective against harmful insect species at high concentrations and short exposure times (Öztekin et al., 2007; Işıkber et al., 2015). Previous studies demonstrated that ozone is an unstable triathomic form of oxygen and the oxygen atoms in this

triple atom structure spontaneously decompose to form hydroxyl radicals and other free radicals or to contact with oxidizable surfaces (Perez et al., 1999). In accordance with our findings, these properties of ozone can provide to prevent bacterial, yeast and mold growth and insect infestations during storage (Xu, 1999).

In addition, both humidity reducer and oxygen absorbent agent, ferric oxide, killed 90 percent of C. lactis females at nearly 9000 mg/L dose within two and half days. The findings obtained from this study are the first evidences on control of C. lactis females with ferric oxide. In accordance with our result, it was reported that ferric oxide is effective on insects in the same phylum (Arthropod) as mites (Janjarasskul and Suppakul, 2018). Previous studies showed that some metal or metal oxides show antibiological properties and act as an oxygen and/or moisture barrier for some bioagents. For this purpose, the some metals such as zinc oxide, silica, titanium dioxide, aluminum oxide and iron oxide are used commercially in food packages (Janjarasskul and Suppakul, 2018). Confirming our results, because of the effects of oxygen-binding of iron oxides, they are commonly used in packaging of fruits as well as many other foods and feeds for control of microorganism and insect infestations and color, odor and ransity changes of fruits (Kavas and Kavas, 2001; Celik and Tümer, 2016; Janjarasskul and Suppakul, 2018; Yıldırım et al., 2018). In this study, nearly 9000 mg/L dose of the other water binding agent, calcium chloride, killed 90% of C. lactis females within three days. Compared to ferric oxide, the agent showed effect on *C. lactis* females at less dose and longest time. But, just the higher dose of silica gel compared to other desiccants killed 90% of C. *lactis* female population approximately after two weeks.

Table 2. The probit analysis and lethal time results of O ₃ , ferric oxide, calcium chloride and silica gel for <i>Carpoglyphi</i>	IS
lactis.	

		03	Ferric oxide	Calcium chloride	Silica gel
Number of experimental time		14	7	10	6
Death rate of control individuals (%)		9.33	6.00	10.23	4
Slope±SE		0.091±0.04	0.095±0.006	0.055±0.03	0.11±0.001
LT ₅₀ (hours)		13.43	48.70	38.84	219.7
95% confidental limits	Highest	11.30	43.04	35.96	200.2
	Lowest	15.97	55.46	42.04	247.1
LT90 (hours)		27.54	62.16	62.09	332.3
95% confidental limits	Highest	23.52	55.41	57.39	294.4
	Lowest	34.13	78.95	68.13	394.8
LT99 (hours)		39.04	73.13	81.05	424.2
95% confidental	Highest	32.78	63.39	74.22	368.3
limits	Lowest	49.63	100.21	90.05	518.2
X ²		233.09	150.41	26.65	45.71
Probability		< 0.01	< 0.01	<0.01	< 0.01

Desiccants such as calcium chloride or silica gel are commonly used to manage humidity in the food packaging headspace. These desiccants are commonly placed into packages in the form of sachets, microporous bags, or are integrated in pads so that they inhibit microbial development (Yıldırım et al., 2018). It was reported that their absorption capacity depends on its water vapour sorption isotherm (Sängerlaubet al., 2013). Differences between the absorption capacities of calcium chloride and silica gel may be caused differences in their lethal doses and lethal times for C. lactis females. Confirming our argument, Singh et al. (2016) showed that some desiccants such as CaCl₂, KCl, and sorbitol, have fast-absorbing capacity with moisture holding capacity $(0.91 \pm 0.01 \text{ [g H}_2\text{O/g]} \text{ in } 120$ h) compared other water binding agents. As a result, our study showed that some moisture-absorbing agents could control C. lactis females at varying doses and times. In this study, the importance of moisture control in combating acarid mites has been shown. Consisting with our study, Solomon (1966) revealed that compared with larger arthropods, the acarid mites have high rates of uptake or loss of water as the percent of weight, but low rates per unit area of its surface. Such arthropods, with a high surface/volume ratio, and living at humidity down to about 62% RH, need a relatively impervious cuticle. Previous laboratory and field studies demonstrated that some inert materials (e.g. silica gel) leading to death through desiccation have proved effective as grain protectants against to some astigmat mites Acarus siro L., Lepidoglyphus destructor (Schrank), Tyrophagus longior (Gervais) and Tyrophagus putrescentiae (Schrank) (Collins, 2006).

As a conclusion, O_3 gas agent has the fastest effect following by ferric oxide, calcium chloride and silica gel, respectively, based on lethal time results. But, there are some disadvantages of the agents in control of *C. lactis*. For example, O_3 can be degraded quickly to O_2 and O atoms. In that reason, the effect of O_3 gas is instantaneous and non-persistent (Güzel-Sevdim et al., 2004). On the other hand, ferric oxide, calcium chloride, silica gel agents could be used only within small volume food packages. The effect of silica gel is very slow. Short term application of O_3 could be suitable for control of the mite in storages. Ferric oxide and calcium chloride could be controlled C. *lactis* during long time. Also, a combined control strategy can be applied against *C. lactis*. Firstly, O₃ gas could be used for fumigation application for 2 days in the storage of dried apricot. After, the dried apricot can be packaged with ferric-oxide or calcium chloride until eating up by consumers. In the future, these combined applications should be tested in producer conditions for reflecting the practice. However, the effects of the agents on microbial load and fruit quantitative should be investigated.

Acknowledgements

This study is a part of Vefa TURGU's MSc thesis which was completed at the University of Bursa Uludağ, Graduate School of Natural and Applied Science, Entomology Department. This study also was presented in XV. International Congress of Acarology 2-8 September 2018 (Antalya-Turkey) as oral, but only was published as abstract in the abstract book of this congress.

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Edited by: Salih Doğan Reviewed by: Two anonymous referees

Citation: Turgu, V. and Kumral, N.A. 2019. Alternative control agents of the dried fruit mite, *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) on dried apricots. Acarological Studies, 1 (2): 59-64.