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Assessment of Naphthalene Residues in Beeswax Foundations Stored in Windscreen Cabinets

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ABSTRACT

Naphthalene has been used by some beekeepers for controlling beeswax moth (*Galleria mellonella*). In this study, firstly, the quantity of naphthalene residues in the beeswax foundations purchased from four different beeswax foundation manufacturers (I, II, III, IV) were determined. Secondly, the wax foundations were attached to frames and kept in the windscreen cabinets for 60, 120 and 180 days. At the beginning, the quantities of naphthalene residues in the wax foundations were measured as 13.67±1.13, 12.95±2.39, 37.68±8.49 and 21.60±3.28 ppb for the manufacturers I, II, III and IV, respectively. After storing the wax foundations for 60 days, the quantities of naphthalene residues were measured as 5.86±0.08, 6.91±0.44, 10.35±2.25 and 8.77±1.29 ppb for the manufacturers I, II, III and IV, respectively ($p<0.05$). Finally, short term aeration of the bee wax foundations reduces the naphthalene level and keeping aeration has additional effects.

Key words: Naphthalene, beewax foundation, storage time, *Galleria mellonella,* aeration

INTRODUCTION

Naphthalene (Npt) is a crystalline, aromatic, white, solid hydrocarbon with formula $C_{10}H_8$. Npt is made from crude oil or coal tar. It is used as an insecticide and pest repellent. It is volatile and sublimes at room temperature and a fumigant insecticide used for various purposes. Npt was formerly used by beekeepers to repel wax moths but has been replaced largely by p-dichlorobenzene. Npt has been used against wax moth (*Galleria mellonella*) prevention by some beekeepers in Turkey. According to an official EU report, Npt was found in exported Turkish honeys to Europe (European Commission, Food and Veterinary Office, 2001). Also, Unal *et al.* (2010) stated that Npt residue found in the honey and beewax collected from different provinces of Turkey. Arslan (2008) reported Npt residue in the only 2 honey samples (included 9.2, 2.8 ppb) from 44 honey samples collected Kars province of Turkey.

Bogdanov *et al.* (2004) reported that residues of Npt were found only in two Swiss honeys but no published reports of Npt residues in honey are available. Primarily, beekeepers worldwide and also in Switzerland, use p-dichlorobenzene (p-DCB) to control the greater wax moth, *Galleria mellonella,* during the storage of beeswax combs (Bogdanov *et al.*, 2004). In Greece, p-DCB and Npt had been used for almost 60 years, until European regulation No. 396/2005 set a limit of 10 $\mu g\ kg^{-1}$ honey for substances where no maximum residue level for honey had been established, including PDCB and Npt (Harizanis *et al*., 2008). Harizanis *et al*. (2008) determined that 46.7 and 78.9% of honey samples collected from different botanical origin contained PDCB and Npt, respectively, in total, 25.6% of the samples were unsuitable for human consumption as either pesticide exceeded the maximum residue limit value.

Both Npt and p-DCB are toxic substances. During the storage of honeycomb, Npt or p-DCB passes to wax, honey and pollen because of sublimation. The accumulated Npt, p-DCB, pesticide and drug residue in honey is harmful to humans. However, the wax containing Npt, p-DCB and pesticides is more dangerous than honey because of its use in several seasons. Due to accumulation of drugs in honeycomb, the drugs pass to honey or pollen and the old honeycombs become a source of potential pesticides in the hive (Wallner, 1995).

Several researchers reported that active substances of the acaricides used especially against varroa accumulate were found in the honeycombs (Lodesani *et al*., 1992; Wallner, 1992, 1995, 1999; Bogdanov *et al*., 1998a; Bernardini and Gardi, 2001), Bogdanov *et al*. (2004) reported that the content of the chemical residue in honey was originated from the recycled usage of honeycombs and the storage conditions. Moosbeckhofer *et al*. (1995) determined that the honey and beeswax could hold pesticide residues for about 10 years.

Wallner (1992) stated that the amounts of accumulated p-DCB quantity in the wax vary depending on air conditioning, room temperature and storage time. Wallner (1992) added that the amount of p-DCB in the wax reduced in the first 2-4 days by ventilation and the content of p-DCB decreased rapidly to 50% and content continued to decrease during the following days. It was reported that wax stored in a cold environment contained high amounts of p-DCB. Bogdanov *et al*. (1998a) determined that the amount of residues in honeycombs was affected by boiling the honeycombs for a long time at high temperatures. The amount of residual acaricides content in unboiled honeycombs was 170% more than the boiled honeycombs.

Wallner (1992) weighed two wax foundations free from p-DCB with an analytical balance and put into a glass jar with 50 g of p-DCB crystals, then the jar was closed airtight and stored at room temperature. Wallner (1999) determined that 1 kg of wax foundation absorbed 37.6 g of p-DCB during a period of 30 days. He had also gassed a wax foundation with p-DCB for 12 d and determined that the wax foundation absorbed 1.94 g of p-DCB and for 12 d of aeration removed 1.80 g of p-DCB from the wax foundation.

In Turkey, the honeycombs are used at least for 2 years and recycled in the beewax factories. Although, Npt usage for bee wax moth control was banned in Turkey some beekeepers have used Npt. Recently most of beekeepers gave up using Npt. But it was not known whether there is Npt residue in recycled wax foundations sold in Turkey. However, since beeswax can hold pesticides for 10 years (Moosbeckhofer *et al*., 1995). The objectives of this research were to determine the Npt residues in beeswax foundations purchased from beeswax recycling factories and to determine the quantity of naphthalene residues in wax foundations stored for 60, 120 and 180 days aerated in windscreen cabinets.

MATERIALS AND METHODS

This study was carried out in 2007 at the Apicultural Unit of Adnan Menderes University in Aydin Province of Turkey. Aydin is located between the 37°44', 38°08' North latitudes and 27°23', 28°52' East longitudes at an elevation of 64 m with subtropical climatic conditions.

In this research, total of 120 wax foundations were bought from 4 licensed factories (30 from each factory) in Turkey. All the wax foundations were attached to the frames and numerated.

Firstly, for each factory total of 30 samples each with 5 cm were thoroughly cut from the bottom sides of wax foundations. Thirty samples were superposed and cut to 3 parts latitudinally. Each part wax sample was covered by aluminium folio separately. Analysis was made to determine the initial Npt residue in wax foundation before storage. Three samples from each factory were analyzed for Npt residue. All the wax foundation frames stored in the windscreen cabinets (surrounded by windscreen on all sides) separately. Thus all wax foundations were exposed to airing. Samples were collected bimonthly from the framed wax foundations for three times. Samples were taken for 60, 120 and 180 days of storage. At each application for each group (factory) determined 10 wax foundation frames by lot. Five centimeter were thoroughly cut from the bottom side again and the same procedure was followed for Npt analysis. Ten wax foundations were superposed and cut to 3 parts latitudinally. Each part wax sample was covered by aluminium folio separately and put into covered glass jars individually and kept in a freezer. After taking the last samples at 180 d, all the samples were analyzed in the Izmir Province Control Laboratory (Republic of Turkey, Ministry of Agricultural and Rural Affairs).

The analyses were performed by SPME-GS-MS. It were used SPME Fiber Assembly (Supelco), GC-MS (Varian 8100, HP-SIM), ultrasonic bath, analytical balance, manual holder (Supelco), graduated screw top Vial (Supelco) autoenjector Napthalene Standards (Aldrich), hexane (Merck), distilled water (Lichrosolv).

One gram wax foundation sample was dissolved in 10 mL hexane in a graduated screw Vial glass was retained for 1 h in the ultrasonic bath. The hexane extract was distinguished from the wax particles by centrifugation for 20 min in a centrifuge, maximum of 22 800 at 4°C. The supernatant was transferred to a new centrifugation tube. The remaining wax components in the supernatant were then removed repeated freezing, subsequent centrifugation and decantation of the clear supernatant as rendered above. Five milliliters of the clear supernatant were added to 20 mL of water ready for SPME. The method used for wax analysis was the same as described by Bogdanov *et al.* (1998b, 2004). SPME: 1.2 mL of the diluted wax solution was extracted into 2 mL auto sampler vial, placed in a SPME auto sampler. Adsorption with a 5 cm 100 μm PDMS-fiber for 45 min at 20-25°C in the liquid phase of the vial, desorption time: 15 min splitless at 250°C. Colon conditions: HP-5 MS, pressure: 75, flow: 1.3 mL min^{-1}, solvent delay: 5 min, library: toxicology, inlet temperature: 200°C.

The data was analyzed statistically by one way ANOVA using MINITAB (13.0) software.

RESULTS AND DISCUSSION

The effects of factory, storage time and the interaction of factory-storage time on Npt residue in wax foundations were found to be statistically significant ($p<0.01$). Overall mean of the residue in the wax foundation was 10.27±1.318 ppb. The results summarized in Table 1 shows that wax foundation produced by four wax factories were contaminated with Npt. The N residue means of factory I and II were below the maximum limit (10 ppb; according to Turkish Food Codex), however the same means were over the maximum limit for factories III and IV. Factory III had the highest Npt residue mean (14.78±4.48 ppb) and this factory was found to be similar to factory IV but different from ($p<0.05$) factories I and II.

The Beginning Naphthalene Quantity (BNQ) mean (21.48±3.66 ppb) was higher than the maximum limit and was also highest in the storage time groups. The differences between the BNQ mean and the storage times were also found to be statistically significant ($p<0.01$). Other differences between the storage times were insignificant ($p>0.05$).

Table 1: The quantity of Naphthalene residue in beewax foundations from factories I, II, III and IV

	BNQ (ppb)		60 d (ppb)		120 d (ppb)		180 d (ppb)		
Factories	N	$\bar{x} \pm s_{\bar{x}}$	N	$\bar{x} \pm s_{\bar{x}}$	N	$\bar{x} \pm s_{\bar{x}}$	N	$\bar{x} \pm s_{\bar{x}}$	Total Aeration* (%)
I	3	13.67 ± 1.13^{ACac}	3	5.86 ± 0.08^{ACa}	3	5.47 ± 0.23^{Aa}	3	5.19 ± 0.18^{Aa}	62.03
II	3	12.95 ± 2.39^{ACac}	3	6.91 ± 0.44^{ACa}	3	5.79 ± 0.16^{Aa}	3	4.82 ± 0.45^{Aa}	62.78
III	3	37.68 ± 8.49^{Bb}	3	10.35 ± 2.25^{ACac}	3	6.37 ± 0.34^{Aa}	3	4.72 ± 0.51^{Aa}	87.47
IV	3	21.60 ± 3.28^{Cc}	3	8.77 ± 1.29^{ACac}	3	7.25 ± 0.86^{ACa}	3	6.91 ± 0.61^{ACa}	68.01
Overall	12	21.48 ± 3.66^{Aa}	12	7.97 ± 0.76^{Bb}	12	6.22 ± 0.29^{Bb}	12	5.41 ± 0.33^{Bb}	74.82

*Npt volatilization after aeration of 180 d, Different uppercase letters denote significant differences at the $p<0.01$ level and different lowercase letters denote significant differences at the $p<0.05$ level

The BNQ means of Npt residue changed significantly during the storage (Table 1). The highest BNQ mean was found for factory III (37.68±8.49 ppb) and this mean was about 4 times higher than the legal limit of Npt residue in beewax foundations. BNQ mean for Factory III was also found to be significantly different from all the storage times and the BNQ means of other factories ($p<0.05$). BNQ mean for Factory IV had the second highest Npt residue and this mean was statistically different ($p<0.05$) from the 120 and 180 d storage times of the factories I, II and III. The quantity of Npt in the wax was started to decrease rapidly at the storage of 60 d and kept decreasing slowly at the storage of 120 and 180 d (Table 1).

In recent years, although most of Turkish beekeepers stopped using Npt, we determined in our study that Npt residues still exist in beeswax foundations produced by recycling factories. In addition, in all wax foundation samples, the Npt residue was found over maximum allowable limits of 10 ppb (European Commission, Food and Veterinary Office, 2001). We also found that the Npt residue levels in beeswax foundations vary significantly depending on the factories. In two factories, we found the Npt residue level over the limits but close to the maximum limit. However, in two factories the residue levels were 2 to 4 times higher than the maximum limit. Due to the recycle of dark honeycombs, the residue of Npt can still be a significant problem for the food safety. Bogdanov *et al.* (2004) informed that p-DCB is not removed from bee wax during the honeycomb recycling process.

As of the end of 1990, in Germany, p-DCB residue level was between 3 and 50 μg in 1 kg of honey in about 50% of German honeys (Wallner, 1992). Bogdanov *et al.* (2004) notified that 30% of honeys produced in Switzerland and 7% of imported honeys in EU had p-DCB residue in between 1997 and 2002.

Finally, toxic substances such as Npt and p-DCB constitutes are serious threats for human health. To get rid of the residues like Npt and p-DCB in beeswax, the only methods are storing the beeswax at room temperature (Wallner, 1992) and boiling of beewax for a long time at high temperature (Bogdanov *et al.*, 1998a).

The toxic effects of Npt or p-DCB can be avoided by adapting alternative methods for *Galleria mellonella* control (Charriere and Imdorf, 1999). The main rules are regular exchanges of old combs with new ones storage of combs under well-ventilated light conditions or at temperatures below 120°C and treatment with non-toxic substances such as sulphur, acetic acid, formic acid or *Bacillus thuringiensis* (Bogdanov *et al.*, 2004; Wallner, 1992).

In our experiments, Npt residues existed in the wax foundations stored at room temperature in the windscreen cabinets. After two months of storage, Npt residues decreased significantly. Storing the beeswax longer (120-180 d) further reduces the Npt residues is but not as much as the reduction observed during the first 60 d of storage.

Like other food products, in the honeybee products food safety concepts is becoming more and more important. Efforts are intended to prevent contamination resulted from the usage of chemicals in honeybee production. Although it is band and not widespread in Turkey, Npt has been used against to beeswax moth (*Galleria mellonella*) during honeycombs storage. However, in this study it was found that short term (60 d) aeration of the beeswax foundations contaminated with Npt residue, residue level decreased significantly and keeping storing the beeswax in the air has additional effects on the decrease of Npt residue.

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REFERENCES

Arslan, I., 2008. Honey produced and sold in Kars and Ardahan regions to investigate the remains of naphthalene. Master Thesis, University of the Caucasus, Kars, Turkey.

Bernardini, M. and T. Gardi, 2001. Influence of acaricide treatments for varroa control on the quality of honey and beeswax. Apitalia, 28: 21-24.

Bogdanov, S., A. Imdorf and V. Kilchenmann, 1998a. Residues in wax and honey after ApiLife VAR treatment. Apidology, 29: 513-524.

Bogdanov, S., V. Kilchenmann and A. Imdorf, 1998b. Acaricide residue in some bee products. J. Apic. Res., 37: 57-67.

Bogdanov, S., V. Kilchenmann, K. Seiler, H. Prefferli and T.H. Frey *et al.*, 2004. Residues of para-dichlorobenzene in honey and beeswax. J. Apic. Res., 43 : 14-16.

Charriere, J.D. and A. Imdorf, 1999. Protection of honey combs from wax moth damage. Am. Bee J., 139: 627-630.

European Commission, Food and Veterinary Office, 2001. Final report of a Mission carried out in Turkey from 8 to October 2001, in order to evaluate the control of residues in live animals and animal products. Report No. 3389, European Commission, Food and Veterinary Office, Brussels, Belgium.

Harizanis, P.C., E. Alissandrakis, P.A. Tarantilis and M. Polissiou, 2008. Solid-phase micro extraction/gas-chromatographic/mass spectrometric analysis of p-dichlorobenzene and naphthalene in honey. Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk Assess., 25: 1272-1277.

Lodesani, M., A. Pellacani, S. Bergomi, E. Carpana, T. Rabitti and P. Lasagni, 1992. Residue determination for some products used against Varroa infestation in bees. Apidologie, 23: 257-272.

Moosbeckhofer, R., K. Wallner, H. Pechhacker, M. Luh and R. Womastek, 1995. Residue level in honey, wax and propolis after ten years of varrao treatment in Austria. Proceedings of the 34th International Apicultural Congress, August 15-19, 1995, Lausanne, Switzerland.

Unal, H.H., H.H. Oruc, A. Sezgin and E. Kabil, 2010. Determined pesticides after honey bee deaths between 2006 and 2010 in Turkey. Uludag Bee J., 10: 119-125.

Wallner, K., 1992. The residues of p-Dichlorobenzene in wax and honey. Am. Bee J., 132: 538-541.

Wallner, K., 1995. The use of varoacides and their influence on the quality of bee products. Am. Bee J., 12: 817-821.

Wallner, K., 1999. Varroacides and their residues in bee products. Apidologie, 30: 235-248.