DERLEME MAKALESİ / REVIEW ARTICLE

IMPACT OF PESTICIDES ON HONEYBEE (*Apis mellifera L.*) DRONES

Tarım İlaçlarının Bal Arılarında (*Apis mellifera L.*) Erkek Arı Üzerindeki Etkileri

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ABSTRACT

Published research about drones is far less extensive than either worker or queen bees because they do not contribute to pollination, brood or honey production. However, much of the reproductive quality of the queen, though, is a function of the mating success and quality of the drones. Besides, studies of drones could help in breeding programs by improving the efficiency and quality of mating. Drones whose reproductive competitiveness is affected by several environmental and in-hive factors during development or adulthood may contribute dead or suboptimal sperm to a queen. It can have severe negative consequences not only for the queen herself but for overall productivity and survival of her colony. Drones are very sensitive to acaricides and insecticides. Most of them have negative impacts not only on drone semen quality such as spermatozoid viability and concentration but also on drone production and their traits.

We here review the studies that describe pesticide exposure that might influence drone fitness.

Key words: Drones, Acaricides, Insecticides, Fitness, Spermatozoa

ÖZ

Erkek arılarla yapılan yayınlar tozlaşma, yavru ve bal üretimine katkı sağlamadığı için işçi ve ana arılardan çok daha azdır. Fakat ana arının üremedeki başarısı kaliteli erkek arılarla başarılı bir şekilde çiftleşmesinin bir sonucudur. Bunun yanında erkek arı ile çalışmalar çiftleşmenin kalitesi ve etkinliğini artırabilir. Erkek arıların üremedeki rekabeti çevresel ve kovan içi gelişme faktörlerinden etkilenir ve bu durum ana arının ölümü veya ideal sperm seviyesinin altında kalmasına neden olabilir. Bu sorun ana arıyı olumsuz etkileme ve dolayısı ile koloninin toplam üretim ve yaşamını çok ciddi derecede olumsuz etkileyecek sonuçlar doğurabilir. Erkek arılar böcek ve akar öldürücü kimyasallara karşı çok hassastırlar. Bu kimysalların çoğu hem erkek arı sperm kalitesi, spermlerin yaşam gücü ve konsantrasyonu ve hem de erkek üretimi ve karakterleri üzerinde olumsuz etkileri bulunmaktadır. Bu ilaçlar erkek arıların yavru döneminde önce işçi arılar ve daha sonra hem işçi arılar ve hemde kendileri kovan içinde beslenirken olumsuz etkilenmektedir.

Bu derleme çalışması tarım ilaçlarına maruz kalan erkek arıların nasıl etkilenebileceği konusundaki çalışmaları kapsamaktadır.

Anahtar Kelimeler: Erkek arılar, Akarisit, Böcek öldürücüler, Uyum, Sperm

GENIŞLETILMIŞ ÖZET

Amaç: Bal arıları ekosistemdeki önemli rolleri ve son yıllarda bal arılarında koloni kayıpları nedeni ile bir çok çalışmanın konusu olmuştur. Bir bal arısı kolonisinde üremeden sorumlu bir ana arı olduğu için ilk akla gelen kolonideki ana arının durumudur. Bu yüzden koloninin geleceği ve üretkenliği için en önemli birey kolonide ana arıdır. Arıcılar ana arıları bu yüzden 1-2 yıl gibi bir sürede değitirmeye çalışırlar. Kolonilerin zayıflamasında ana arının yetersiz veya düşük kaliteli erkek arılar ile çiftleşmesi ana etkenlerden biridir.

Tartışma: Erkek arılar ticari olarak önemli olmadığı ve kısıtlı bir zaman diliminde üretildiği için işçi arılar kadar yoğun olarak çalışılmamıştır. Erkek arılar zamanın çoğunu kovan içinde geçirsede özellikle larval dönemde ve daha sonra böcek öldrüücü veye tarım ilaçlarına maruz kalabilmektedir. Dolayısı ile erkek arılar hem arıcıların kovan içinde özellikle varroa parazitine karşı kullandığı ve çevredeki tarım ilaçlarından etkilenebilmektedir. Bu durumda varrao için kullanılan sentetik ilaçlar, organic asitler ve esansisyel yağlar gibi ilaçlar kullanılmaktadır. Bunun yanında bazı ülkelerde nosema ve yavru çürüklüğü hastalıkları için antibiyotikler de kullanılabilmektedir.

Erkek arılarla yapılan yayınlar tozlaşma, yavru ve bal üretimine katkı sağlamadığı için işçi ve ana arılardan çok daha azdır. Fakat ana arının üremedeki başarısı kaliteli erkek arılarla başarılı bir şekilde çiftleşmesinin bir sonucudur. Bunun yanında erkek arı ile çalışmalar çiftleşmenin kalitesi ve etkinliğini artırabilir. Erkek arıların üremedeki rekabeti çevresel ve kovan içi gelişme faktörlerinden etkilenir ve bu durum ana arının ölümü veya ideal sperm seviyesinin altında kalmasına neden olabilir. Bu sorun ana arıyı olumsuz etkileme ve dolayısı ile koloninin toplam üretim ve yaşamını çok ciddi derecede olumsuz etkileyecek sonuçlar doğurabilir. Erkek arılar böcek ve akar öldürücü kimyasallara karşı çok hassastırlar. Bu kimysalların çoğu hem erkek arı sperm kalitesi, spermlerin yaşam gücü ve konsantrasyonu ve hem de erkek üretimi ve karakterleri üzerinde olumsuz etkileri bulunmaktadır. Bu ilaçlar erkek arıları yavru döneminde önce işçi arılar ve daha sonra hem işçi arılar ve hemde kendileri kovan içinde beslenirken olumsuz etkilemektedir.

Sonuç: Tarım ilaçları ve kovan içinde başta varroa için kullanılan akar ve böcek öldürücü ilaçlar, organik asitler, esansiyel yağlar erkek arılarda önemli etkilere neden olmaktadır. Son yıllarda yapılan çalışmalarda yeni nesil tarım ilaçları olan neonikotinoidlerinde erkek arılar üzerinde olumsuz etkilere sahip olduğu rapor edilmiştir. Bu ilaçların erkek arıların yaşam süresi, doğum ağırlığı, kanat boyu, genişliği, uçuş faaliyetleri, sperm miktarı ve kalitesi üzerinde olumsuz etkileri olduğu yapılan çalışmalar sonucunda görülmektedir. Ayrıca bazı tarım ilaçları spermlerin yaşam gücünü ciddi derecede düşürmektedir.

Bal arıları her ne kadar dişi merkezli canlılar olsada erkek arıların üremedeki başarısı doğal seleksiyon açısından ana etken olarak görülmekte olduğundan çevre faktörlerinin erkek arıların sağlığını nasıl etkilediğini belirlemek önemli bir konu olarak düşünülmektedir.

INTRODUCTION

Honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), play a highly complex and significant role in the ecosystem. They have been the focus of many studies in recent years due to the progressive decline in their number (Neumann and Carreck 2010, Potts et al. 2010, Goulson et al. 2015). Pathogens and pesticides are thought to weaken the immunity of bees and affect their physiology and development (Frazier et al. 2008). To determine the main causes of the high rate of weakening in honey bee colonies, special attention has been focused on queens (Traynor et al. 2016). The queen is the only reproductive female of the colony and is the main key when assessing colony reproductive output (Winston 1987) and a principal focus of beekeepers. Although queens have a 3–4 year adult lifespan (Winston 1987), commercial beekeepers typically replace their queens every 1–2 years because of the critical importance of a vigorous queen to colony survival and productivity (Amiri et al. 2017). Multiple drones are required to inseminate the queen with high-quality sperm on one or more mating flights (Woyke 1955). After the mating, the queen will store the sperm in her spermathecae and fertilize eggs. Queens generally mate with approximately 12–20 drones (Tarpy et al. 2015) and up to 45 drones (Neumann and Moritz 2000). Queens inseminated with sub-fertile drones are themselves reproductively impaired. Thus, while “poor queens” may be a leading reason for colony failure, a major cause of queens being perceived as poor is very likely to be “poor drones” (Kairo et al. 2016).

Drone Reproductive Development

Compared to workers honeybee, drones have not been thoroughly investigated because they are not of direct commercial interest and they are reared only during limited periods. Drones live less than workers who live about 40–140 days depending on the season. Drones can live until 60 days (Page and Peng 2001) but some studies show that they can reach 90 days (Fukuda and Ohtani 1977). They either die during mating or killed by workers when the swarming season comes to an end.

The development of drones from egg stage until emergence took approximately 24 days in larger cells compared to those of workers (Smith et al. 2014). The reproductive biology of male honey bees is distinct from that of mammals. In drones, sperm production starts in the testes during pupation and the sperm migrate to the seminal vesicles during the adult drone maturation, which is reached at least 16-18 days after adult drone emergence (Metz and Tarpy 2019). The migration of sperm to the seminal vesicles starts two days before eclosion. Spermatozoa and the entire body of drones have to undergo yet a physiological maturation process where the secretion of the glands of the genital tract is essential (Snodgrass 1984). Nutrition can affect the timing of drone sexual maturation (Rhodes 2008). Many factors (environmental and biotic) can also affect drone reproductive quality such as age, season, and genetics (Rhodes et al. 2010).

Mature drones are most of the time in the hive. They are either resting, feeding or cleaning themselves (Fukuda and Ohtani 1977), and flights occur when conditions are favorable, around afternoon (Ruttner 1966). Drones make on average 2–4 flights per day and fly up to 7 km from their hive. Orientation flights last approximately 1–6 min, whereas mating flights take about 20–30 min (Currie 1987).

How Drones can Be Exposed to Pesticides?

Recent studies focused on the exposure ways of pesticides on honeybee pollination services (Goulson et al. 2015) with residues in nectar and pollen (Mullin et al. 2010), surface water (Wauchope 1978) and floral secretions and plant exudates (Girolami et al. 2009). Honeybees store these products in the hive leading to exposure of brood and hive products (Ravoet et al. 2015).

Trophallaxis, the exchange of food by mouth, occurs among colony’s castes. Drones are fed by workers of all ages frequently (Haydak 1957). During their development, drone larvae receive more food than worker larvae (avg. 9.6 mg versus 1.7 mg per cell) (Haydak 1957). For the first few days of their lives, drones are fed entirely by workers. Later, they are both fed by workers and feed themselves from honey cells. The larval diet which is composed mainly of pollen and nectar given to honey bee larvae expose them transdermal, orally and internally; therefore, the potential chronic toxicity and synergistic interactions at the brood stage seems likely to occur, knowing that life stages might be much more sensitive to certain contaminants compared to the adult stage (Zhu et al. 2014)

Impact of Pesticides on Drone Fitness

The process of finding a queen and mate with her involves the capacity of drones to be able to reach a drone congregation area (DCA) (Koeniger et al. 2014), locate the queen, compete with thousands of other drones, and deliver sperm that the queen will store in her spermatheca (Winston 1987). Hence, the ability to copulate which involves the body size (Berg et al. 1997) and the ability to inseminate are critical in order for a drone to offer a genetic diversity passed to the next generation.

Drones can be exposed to a large set of chemicals from their environment and from beekeeping practices. Agricultural activities can be a source of a variety of pesticides. But also the products administered to bee colonies by the beekeeper are at least equally noteworthy. Indeed, beehives are treated against the ectoparasitic mite *Varroa destructor* with many chemicals, like pyrethroid and organophosphate acaricides. Also naturally occurring organic acids and essential oils are used (Rosenkranz et al. 2010). Further, different antibiotics are commonly used in some countries for preventive or therapeutic treatment against American and European foulbrood, and against nosemosis (Reybroeck et al. 2012).

Synthetic compounds such as coumaphos (Check-Mite™ or Perizin®), fluvalinate (Apistan®, Gabon®), flumethrin (Bayvarol®) and amitraz (Apivar®, Varidol®) are the main used chemicals to control *V. destructor*. In order to find more “natural” treatment against varroasis, oxalic acid, formic acid (Formidol®) and thymol (Thymovar®) have been introduced and are becoming increasingly used in organic beekeeping.

Few studies focused on the impact of pesticides drones traits such as body weight and wings length and width of drones. Rinderer et al. (1999) were the first to study the effect of fluvalinate (the active ingredient in the product Apistan®) on drone production. They found that mortality was higher in fluvalinate-treated colonies (66.9%) in drones aged between 12 and 18 days old, compared to control. They also reported that surviving drones from colonies treated with fluvalinate caused drones to have about 5% reduction in body weight and the interaction of *Varroa* and fluvalinate led to 10% reduction of drone body weight. Drones from coumaphos treated colonies presented lower viability in the first week (86%) compared to drones from untreated colonies (90%). The viability reached the lowest rate by week 6, with a percentage of 49% compared to control (85%) (Burley et al. 2008). Shoukry et al. (2013), treated emerged drones with different miticides. They found a reduction in wing length ranged from 5.36% in drones treated with fluvalinate compared to control drones. Also, fluvalinate and amitraz treatments significantly decreased the wing width of drones by approximately 4.57% and 2.27% respectively compared to the control.

The impact of organic treatments on drone survival and traits were also investigated by De Guzman et al. (1999). Treated colonies with formic acid produced less than half of drones than untreated colonies. A reduction of the survival of ten days old drones and a reduction of wing length were also reported. Drones treated with formic acid and oxalic acid induced a reduction in wing length and a reduction of 2.31% and 4.52%, respectively (Shoukry et al. 2013). Furthermore, a high percentage of oxalic acid (more than 0.5%) reduced the survival of the drones (Aboushaara et al. 2017). Sublethal doses of thymol treatment caused a reduction in drone flight activity (Johnson et al. 2013).

Given the reproductive quality of drones, most of the studies were focused on the impact of acaricides and insecticides on drone fertility and semen quality. In fact, coumaphos caused reduced sperm viability immediately after semen collection and in samples stored up to 6 weeks (De Guzman et al. 1999). Burley (2007) explored the effects of Apistan® (fluvalinate), Checkmite+® (coumaphos), or Apilife Var® on sperm number and viability in the seminal vesicles of mature drones. The lower sperm viability was found in coumaphos treated colonies. Besides, drone sperm number was lower in all treated colonies.

Fisher and Rangel (2018) investigated the effects of the drone-rearing beeswax exposed to miticides (amitraz with a mix of coumaphos and fluvalinate) and agrochemicals (chlorpyrifos and chlorothalonil) found in hives. Mean sperm viability was significantly lower in drones reread in all treated beeswax. They also found that sexual maturity of treated drones groups reached when they were aged between 16 to 18 days of age.

Shoukry et al. (2013) also evaluated the effects of two acaricides (fluvalinate, amitraz), two organic acids (oxalic acid, formic acid) and thymol on drone fertility. The lowest sperm number was found in drones exposed to fluvalinate and amitraz, while a concordance was observed between sperm number and wing length among all treatment groups.

Besides acaricides, several agrochemicals have been found to negatively affect the drone semen quality. Oral exposure to the neonicotinoid insecticides such as thiamethoxam and clothianidin are known to reduce sperm viability in adult drones (Straub et al. 2016). Chronic exposure of sublethal doses of clothianidin at sexual maturity stage decreased semen volume and its concentration and increased sperm mortality rate (Ben Abdelkader et al. 2018). It also induced oxidative stress in spermatozoa by increasing antioxidants enzymes (superoxide dismutase, glutathione peroxidase, and catalase) and malondialdehyde level, which is a lipid peroxidation marker, in spermatozoa of drones exposed and also decreasing the protein content in semen (Ben Abdelkader et al. 2019).

Exposure to imidacloprid was also found to affect the mitochondrial activity of spermatozoa and therefore the sperm viability (Ciereszko et al. 2017). Phynelperazole insecticides also affected the drone reproductive system. Sublethal doses of fipronil at sexual maturity led to a decrease of spermatozoa concentration, sperm viability and an increase in sperm metabolic rate (Kairo et al. 2016). The co-exposure of fipronil with the microsporidian parasite *Nosema ceranae* induced metabolic disturbances and affect oxidative stress defense in sperm.

Moreover, the acute *in vitro* exposure of six molecules (fipronil, ethiprole, imidacloprid, thiamethoxam, cypermethrin, and coumaphos) of spermatozoid at different concentrations ranged from 0.1 to 100 μM led to an increase of spermatozoid ATP levels. Fipronil, ethiprole, imidacloprid and thiamethoxam significantly decreased the viability of spermatozoids (Ben Abdelkader et al. 2015) in 24 hours.

CONCLUSION

Managed honey bee colonies are exposed to multiple pesticides including insecticides such as neonicotinoids widely used in the foraging environment or miticides used to control *Varroa* mites, which are still widespread in hive products and larvae food, can negatively impact drone fitness. Acaricide treatment negatively affects the drone and mucus glands weight, the spermatozoa number, the sperm viability and seminal vesicles weight. Exposure food to commonly used systemic insecticides had shown significant negative effects on drone reproductive quality by significantly reducing the sperm viability and concentration.

Although the life cycle of honeybee colonies is highly dependent on females, the male reproductive fitness appears to be a key driver of natural selection in honeybees. Hence, it is important to understand and find out more about how environmental factors could affect their health.

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