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In vitro efficacy and field effectiveness of silica-based acaricide against *Dermanyssus gallinae*

Dermanyssus gallinae mücadelesinde silika bazlı akarisitlerin in vitro etkinliği ve saha koşullarında etkililiği

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ABSTRACT

Objective: The poultry red mite, PRM, *Dermanyssus gallinae* (*D. gallinae*), is one of poultry's most important and common pests. Although the presence of a variety of acaricides in control, PRM continues to be frequently reported around the world, owing to the developing resistance and severe legislation prohibiting the use of chemicals. Therefore, new and safe control approaches that do not threaten animal and human health are required. This study is aimed to determine the efficacy of a natural silica-based product based on diatomaceous earth (DE) in-vitro conditions and its combination with locally applicated synthetic pyrethroids under field conditions in commercial egg-laying henhouses.

Methods: *Dermanyssus gallinae* populations were collected and transferred alive to the laboratory from integrated cage poultry farms. The bioassays were performed with the liquid formulation of silicon dioxide. The LT_{50} values were calculated by probit analysis using PoloPlus-PC software. The field treatment was conducted using the combination of silicon dioxide and alpha-cypermethrin in two commercial poultry houses with natural PRM infestation. After application, mites were collected with PVC traps on different days. The traps were individually packed and the number of mites

ÖZET

Amaç: Kırmızı kanatlı akarı (KKA), *Dermanyssus gallinae* (*D. gallinae*), kanatlı sektörünün en önemli ve yaygın görülen ektoparazitlerinden biridir. Mücadelesinde kullanılan çok sayıda akarisit olmasına rağmen gelişen direnç ve kimyasalların kullanımını yasaklayan mevzuat nedeniyle KKA enfestasyonu tüm dünyada sıklıkla bildirilmeye devam etmektedir. Bu nedenle insan ve hayvan sağlığını tehdit etmeyen yeni ve güvenli kontrol metotlarına ihtiyaç duyulmaktadır. Bu çalışmada, in vitro koşullarda doğal silika bazlı akarisitlerin etkinliğini ve ticari yumurta tavuğu kümeslerinde lokal olarak uygulanan sentetik piretroidler ile kombinasyonunun etkinliğini belirlemek amaçlanmıştır.

Yöntem: *Dermanyssus gallinae* popülasyonları, kafesli, ticari yumurta tavuğu kümeslerinden toplanmış ve canlı olarak laboratuvara getirilmiştir. Laboratuvar denemeleri sıvı formülasyon haline getirilen silikon dioksit ile gerçekleştirilmiştir. LT_{50} değerleri PoloPlus-PC programı kullanılarak probit analizi ile belirlenmiştir. Saha uygulaması, doğal olarak KKA enfestasyonu bulunan iki ticari kümeste silikon dioksit ve alfa-sipermetrin kombinasyonu kullanılarak yürütülmüştür. Uygulama sonrası belirli günlerde kurulan PVC tuzaklar ile akarlar toplanmıştır. Bireysel olarak paketlenip laboratuvara

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was counted under a stereo microscope in the laboratory. The efficiency of the application was then determined by counting the mites under a stereomicroscope.

Results: In laboratory conditions, DE active substance at the concentration of 320 mg/dish eventually reach 100% mortality of *D. gallinae* and the mean LT_{50} values were determined as 26.42±0.57 h. The combination of silicon dioxide and alpha-cypermethrin in field conditions was found to be highly effective in reducing the number of PRMs and its effects lasted for four months following treatment.

Conclusion: These results suggest that DE might offer an environmentally-friendly alternative in PRM control on its own or in combination with widely used synthetic pyrethroids.

Key Words: *Dermanyssus gallinae*, The poultry red mite, silicon dioxide, management

getirilen tuzaklar stereo mikroskop altında incelenmiş ve içerisinde bulunan akarların sayımları yapılarak uygulamanın etkinliği takip edilmiştir.

Bulgular: Laboratuvar koşullarında 320 mg/petri konsantrasyonunda kullanılan silikon dioksit, *D. gallinae* üzerinde %100 ölüm oranı oluşturmuş ve ortalama LT_{50} değeri 26.42 ± 0.57 s olarak hesaplanmıştır. Saha koşullarında ise silikon dioksit ve alfa-sipermetrin kombinasyonunun KKA sayısını azaltmada oldukça etkin olduğu ve etkisinin uygulamayı takiben dört ay sürdüğü tespit edilmiştir.

Sonuç: Laboratuvar koşullarında uygulanan doğal silikon dioksit ve kümeslerde lokal olarak uygulanan piretroid ile kombinasyonlarının, *D. gallinae*'yi kontrol etmek için etkili bir seçenek olabileceği görülmüştür.

Anahtar Kelimeler: *Dermanyssus gallinae*, kırmızı kanatlı akarı, silikon dioksit, mücadele

INTRODUCTION

The ectoparasite, poultry red mite (PRM), *Dermanyssus gallinae* (De Geer, 1778) has a global distribution with a prevalence rate of over 80% among European layer farms (1), including Turkey (2, 3). It is known as one of the most harmful blood-sucking ectoparasites and its infestation causes significant welfare problems, both directly and indirectly through its role as a vector of infectious disease-causing pathogens (4-6) as well as great economic losses, notably in the egg-laying industry (7), amounting to 231 million euros in Europe (8).

The control of the PRM is primarily based on chemical methods, many of which are not safe for hens, humans, and ecosystems (9, 10). Organophosphates, amidines, carbamates, isoxazolines, and synthetic pyrethroids are still routinely used for the management of *D. gallinae* in egg-laying farms (7, 11). However, the strict legislations about residues threatening human health on products and the increasingly developing resistance causing failure in control, conclude a discouraging effect for the farmers to avoid chemical strategies (9, 12, 13).

Because of the above-mentioned reasons, new and safer control methods such as vaccination, predatory mites, entomopathogenic fungus, essential oils, and organic acids are required (14, 10). Silica-based products containing silicon dioxide (SiO_2) constitute one of the remaining control options against arthropod pests (15). Silica's acaricidal effects have been attributed to a variety of mechanisms, including affecting the digestive system, blocking the stigmas and trachea, absorbing lipids in the cuticle, and destroying the protective waxy barrier (16). Therefore, the arthropods could not able to develop resistance to such products (17). The impact of these products varies depending on the silica quality, its ability to hold water, environmental factors, and the degree of bonding to the treated surfaces (14, 18). Studies have shown that silica products, both in liquid and powder form, have been proven to have a substantial acaricidal effect. Additionally, the acaricidal effect also was reported both in natural (diatomaceous earth (DE))

and synthetic (amorphous) silica products (15). That's why silica and its combinations have a lot of promise for future non-chemical *D. gallinae* management.

The present study aimed to determine the performance of a natural silica-based product based on DE in-vitro conditions and to indicate the effectiveness of silica combined with topical application of synthetic pyrethroids to cage equipment under field conditions in commercial egg-laying henhouses.

MATERIAL and METHOD

Dermanyssus gallinae populations were collected from integrated cage poultry farms located in Turkey. The sampled mites were subsequently transferred to the laboratory for the experiment in nylon bags containing a paper towel to reduce the humidity inside and allow hiding places for the mites. Totally, the experiments were performed with three replicates, on three different *D. gallinae* populations.

Bioassays

The experiments were conducted under controlled climatic conditions (25±1 °C, 65-70% relative humidity, 16:8 h light-dark photoperiod). For the bioassays, semi-engorged adult female mites were separated and housed in laboratory conditions for 48 hours before being analyzed. All bioassays were performed with mites on filter paper (surface area: 16cm^2) found in glass petri dishes (diameter: 9 cm).

A commercial formulation of silica including 100% diatomaceous earth as an active ingredient was used in the experiments. The powdery test substances were combined with distilled water to prepare the fluid products. The mixtures were taken for bioassays onto filter paper that was placed in petri dishes. The active substance was 320 mg/dish. Subsequently, the edges of the filter paper were covered with the glue trap in order to prevent mites from escaping the experimental area. Then, 30 adult female mites were transferred onto the filter paper. Mortality was recorded at least twice until all mites died and mites that could not move when touched with a fine brush were deemed dead. The control groups were sprayed with distilled water, and the controls' mortality rates were consistently less than 10%. Lethal Time (LT), LT_{50} values, and their 95% confidence limits were determined by probit analysis using PoloPlus-PC (LeOra, Software, Berkeley, CA, USA). The results have been considered significantly different ($p>0.05$) if the confidence limits did not overlap (19).

Field Trial

The treatment was performed at two commercial poultry houses with a spontaneous PRM infestation history. The houses had cage systems containing 120.000 egg-laying hens. During the field treatment, the temperature and relative humidity of the poultry houses were recorded as 21°C and 53%, respectively.

Liquid preparation of Silicon dioxide was applied to whole henhouses at the recommended dose (20 gr/m^2). The pyrethroid, alpha-cypermethrin, was also performed exclusively in the gap found in the cage rows without contacting hens to prevent leaving residues in eggs or tissues. Before the treatment, the number of mites was first determined using PVC (polyvinyl chloride) traps as described in Koç and Nalbantoğlu, (2021). The mite counts were also recorded after 7, 30, 60, and 90 days of applications. After 48h of each trap being placed, the traps were kept separate in plastic bags, then delivered to the laboratory to be counted for *D. gallinae*.

RESULTS

Bioassays

The results of the bioassays are presented in Table 1. The LT_{50} values of PRM1, PRM2, and PRM3 were detected as 25.95, 26.1, and 27.23, respectively. Mite mortality reached 100% within 48h after treatment under laboratory conditions.

Field Trial

During the treatment period of four months, a similar effect on the PRM population under field conditions was observed in two different henhouses (Figure 1). The combination of silicon dioxide and alpha-cypermethrin was found to be highly effective

for reducing the number of PRMs during the first two weeks post spraying. In the second week, following the treatment, the average number of PRM were 260 and 480 for Cage1 and Cage2, respectively. In addition, an increase in the mite number was detected in the second month and it was recorded that the treatment was required after about four months. As a result of multi-residue analysis performed routinely, alpha-cypermethrin residues were not detected in egg samples (personal communications with the henhouses' official veterinarian).

Table 1. LT_{50} values of silicon dioxide in field-collected populations of *Dermanyssus gallinae* (determined by probit analysis)

Populations	LT_{50} (h) (95% CL)	Slope + SE	chi^2	*p*
PRM1	25.95 (21.148-31.120)	3.597+0.511	8.9645	0.2552
PRM2	26.1 (21.003- 31.490)	3.941+0.537	10.524	0.1607
PRM3	27.23 (22.410- 32.344)	4.079+0.550	8.9894	0.2534

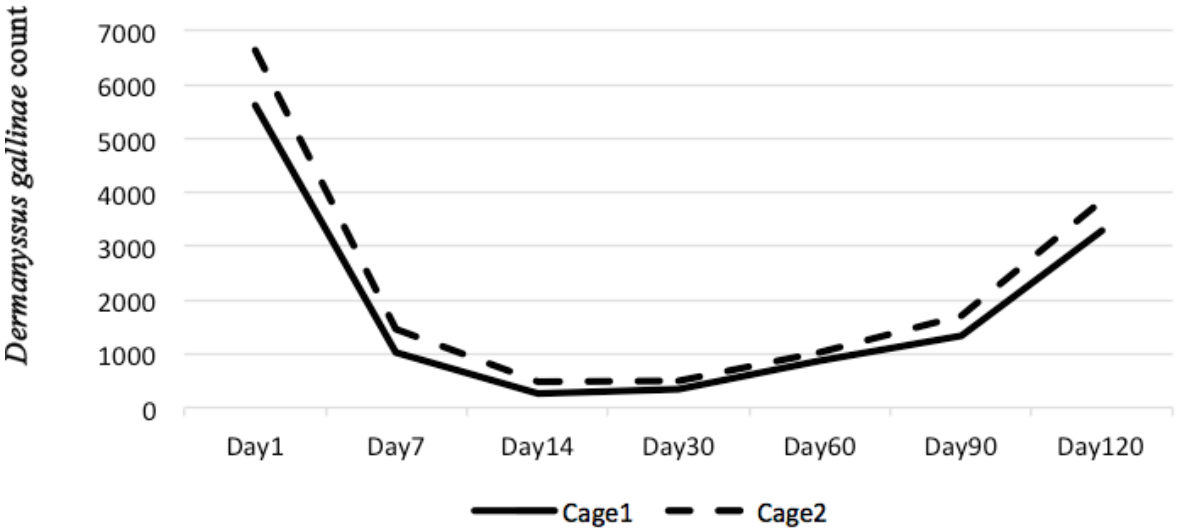

Figure 1. FThe effect of silicon dioxide and alpha-cypermethrin treatment over time on the presence of *Dermanyssus gallinae* in henhouses

DISCUSSION

Chemical pesticides are indeed considered to be the quickest and easiest tools to control the poultry red mites in henhouses. However, developing resistance against synthetic acaricides (12, 13) and chemical residue found in poultry products (9, 20) leads to failure in the control of mites and therefore, *D. gallinae* will become a growing threat to global egg production. As a result, more effective and safer approaches for controlling PRM are necessary. This study investigated the efficacy of a natural silica-based product in-vitro and in the field when combined with synthetic pyrethroid.

The result of bioassays showed that PRM mortality reached 100% within 48h after treatment at the recommended dose and the mean LT_{50} values were

determined as 26.42±0.57 on adult mites. The other experimental studies using similar silica-based products reported high LT_{50} values, 31.7 and 34.9 at 25 °C, with 80% relative humidity (21). This can be explained by the effect of the humidity difference. Because the efficacy of silicas is inversely associated with relative humidity. These silicas lose their potency at greater relative humidities (16, 18, 22). Besides adults, silica-based products can affect all developmental stages of mites. Although its effect on eggs is controversial, the hatched larva died one hour after coming into contact with silica. Therefore, the larvae could be considered to be more sensitive than eggs. On the other hand, a significant difference between the larvae and adult mortality when treated with silica was not observed (18).

In the field experiment, a gradual reduction in the mites populations over time was detected in accordance with Alves et al., (2020), which applied liquid silica followed by mechanical cleaning. A previous study reported a 53.5% reduction rate 14 days after silica application in field conditions (23), whereas an approximately 95% decrease in the number of mites was detected on the 14th day in this study. The increased efficacy could be related to combining alpha-cypermethrin application. To date, combinations of silica-based products and the fungus *Beauveria bassiana* (24, 25), as well as, mechanical cleaning (23) have been assessed. The mortality of the mixture of conidia and DE was recorded as up to 38% higher than in separate applications (24). Mechanical cleaning performed before the application of DE increased the efficacy of the treatment (23).

In conclusion, DE liquid preparations have great potential to reduce the number of *D. gallinae* in henhouses. However, the efficacy remains incapable unless used on its own. Therefore, different combinations are needed to enhance the impact of silicas. In the current study, it was observed that the combined use of the silica-based product with the topical application of alpha-cypermethrin increased the effectiveness. In addition, the application of alpha-cypermethrin on the cage equipment would take advantage in preventing the chemical residue in poultry products. However, further studies are necessary to optimize the combination's practical use, and the eggs should be checked again for alpha-cypermethrin residue.

ETHICS COMITTEE APPROVAL

* This study does not require Ethics Comittee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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