Effect of some essential oils (*Allium sativum* L., *Origanum majorana* L.) and ozonated olive oil on the treatment of ear mites (*Otodectes cynotis*) in cats

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Abstract: The purpose of this study was to determine the effect of certain essential oils and ozonated olive oil on *Otodectes cynotis* infestations in cats. Twenty-eight cats were included in the study. Infestation was diagnosed through the microscopic examination of ear secretions. Clinical findings were evaluated and scored before and after treatment. Cats were randomly assigned to different treatment groups, which included Group 1 treated with ozonated olive oil, Group 2 treated with garlic oil, Group 3 treated with marjoram oil, and Group 4 treated with permethrin. Each treatment was applied to both ears with approximately 5 drops daily per ear for a period of 10 days. The effectiveness of treatments was determined using an efficacy formula. By day 10, G1 and G4 were the groups showing the highest effectiveness. By day 30, effectiveness between the groups ranked as follows: G4 > G1 > G3 > G2. Based on these results, it can be stated that garlic, marjoram, and ozonated olive oil represent cheap, easily applicable, and safe alternatives to conventional treatments with no side effects for *O. cynotis* infestations in cats.

Key words: *Allium sativum* L., ear mite, essential oils, *Origanum majorana* L., *Otodectes cynotis*, ozonated olive oil

1. Introduction

Otodectes cynotis is an ectoparasite affecting cats, dogs, foxes, and weasels and is a highly contagious and frequently encountered parasitic infestation in cats. *O. cynotis* is regarded as a potentially zoonotic disease, which makes it even more significant (1). The parasite is easily transmitted through contact with an infested animal, and although it can affect all age groups, it is observed more frequently in the offspring of infested mothers. It is also reported that cats are the main agent in transmission to dogs (1–3). *O. cynotis* settles on the epidermal surface of the outer ear canal and feeds on epithelial remnants, blood, and tissue fluids (3). The infestation has a fairly characteristic appearance, with the outer ear canal being filled with various amounts of a dry, dark red-brown substance (2). Among infested cats, 85.4% exhibit abnormal ear secretions, while 41.5% suffer from mechanical irritation caused by the parasite and itching due to allergens and toxic substances in tissues. *O. cynotis* causes an intense irritation in the ear canal, resulting in otitis externa. Otitis externa is observed in nearly 50% of all cats, while *O. cynotis* has been identified in the ear discharge of up to 84% of cats (4–6). If left untreated, otitis externa can lead to serious complications including bacterial and fungal infections. In severe cases, ear hematoma, nodding, incoordination, ataxia, bleeding, deafness, epilepsy, and perforated eardrums have also been reported (7,8).

O. cynotis treatment involves various drugs such as lindane, benzyl benzoate, carbaryl, pyriproxyfen, thiabendazole, monosulfiram, permethrin, ivermectin, selamectin, imidacloprid, moxidectin, and fipronil (9–11). However, all the products used in conventional treatment have various side effects (1,12,13). Other disadvantages include difficulty in application, lengthy administration periods (14), health risks for individuals administering the treatment, undesirable effects on the environment (15), and high cost. Current substances used for *O. cynotis* treatment are not licensed and are thus used off-label, making follow-up difficult. The possibility of resistance increases due to unnecessary and repetitive applications of these antiectoparasite drugs designed for once-a-month conventional treatment of ectoparasites such as lice, fleas, and ticks (1,14,16). Since these drugs are readily available, they can be used by cat owners at any time with little awareness of their hazardous effects. While these products are declared as safe for animals from a toxicological

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standpoint, there can nevertheless be serious health risks for animal owners as a result of exposure and contamination during application, an issue that is often overlooked. Veterinarians, in particular, are more likely to be exposed to contamination during the application of these drugs and are consequently under significant risk (15,17). Furthermore, conventional antibacterial, antifungal, antiinflammatory/ analgesic, and antiectoparasite preparations used for the treatment of otodectic mange may also potentiate drug resistance when used unnecessarily in cases where otitis is nonexistent. In addition, ototoxic antibacterial and antifungal agents should not be used in cases involving perforated eardrums. The disadvantages associated with conventional treatments make it necessary to search for more reliable substances. In this context, the purpose of this study was to find alternatives that would overcome the aforementioned disadvantages (18).

A trend exists in research with regard to the development of alternative products such as essential oils for ectoparasite management in veterinary medicine. Essential oils may be used for alternative treatment of mite infestations because they are rich in bioactive chemicals determined to have insecticidal and acaricidal effects (19,20). This has led pet owners in developed countries to begin to prefer more affordable and reliable plant-derived drugs for the treatment of *O. cynotis* infestation, largely due to the harmful side effects and persistent residues associated with the conventional drugs (18).

In this study, the effects of ozonated olive oil, garlic oil (*Allium sativum* L.), and marjoram oil (*Origanum majorana* L.) on *O. cynotis* infestations in cats were investigated and compared with the intention of identifying an alternative to conventional treatments such as permethrin. This study is the first to establish the effect of topical use of ozonated olive oil against *O. cynotis* infestation.

2. Materials and methods

2.1. Selection of animal material
The study included a total of 28 cats of both sexes (10 females and 18 males) of various ages (aged between 2.5 months and 5 years) and different breeds (1 Iranian, 1 Ankara, and 26 crossbreeds) admitted to the Animal Hospital of the Afyon Kocatepe University Faculty of Veterinary Medicine and private veterinary clinics with clinical complaints such as severe itching of the head and ear regions, alopecic patches, and abnormal brown ear secretions. The study was approved by the Institutional Animal Care and Use Committee of Afyon Kocatepe University. Written consent concerning the participation of their pets in the study was obtained from cats' owners.

2.2. Parasitological examination
The external ear canals of all cats were examined using an otoscope. Ear secretions were collected with a swab from cats suspected of having *O. cynotis* infestation. The secretions were then placed on a glass slide and examined under a light microscope after applying a few drops of mineral oil (10× and 40×). The presence of *O. cynotis* was determined based on microscopic examination (1,21).

2.3. Substances applied to the study groups
Ozonated olive oil (Good & Health, Mert-Koz Cosmetic Ltd., İstanbul, Turkey), essential oil of *Allium sativum* L. (Defne Essencia, NUKA Ltd., Antalya, Turkey), *Origanum majorana* L. (NBT Life Natural Products Ltd., Antalya, Turkey), and permethrin-containing solution (10 mg/g) (Oridermyl, Vétoquinol N.-A., Lavaltrie, Canada) were applied to the cats.

2.4. Gas chromatography analysis
The amount of active substances/ingredients in the essential oils used in this study (*Origanum majorana* L. and *Allium sativum* L.) was analyzed at the Central Laboratory of Mustafa Kemal University using gas chromatography-mass spectrometry (GC/MS).

2.5. Grouping of the infested cats and application of active substances
Twenty-eight cats infested with *O. cynotis* were equally divided into four groups. Group 1 (G1) was the ozonated olive oil-administered group, Group 2 (G2) was the *A. sativum*-administered group, Group 3 (G3) was the *O. majorana*-administered, and Group 4 (G4) was the permethrin-administered positive control group. All groups were otoscopically examined for eardrum integrity; following this examination, a treatment of 5 drops (0.3 mL) once daily in both ears for a period of 10 days was administered to cats. After each application, the ears were massaged for 60 s to ensure sufficient penetration of the substance into the external ear canal. No acaricidal drug was used for pretreatment. Cats were kept in individual cages during the treatment and checked for control after treatment (days 10 and 30).

Parasitological examination results as well as clinical findings (such as erythema, ulceration, pruritus, pain, amount of secretion, and secretion types) observed during physical and otoscopic external ear canals examinations were recorded before and after treatment. Live *O. cynotis* parasites observed under a microscope prior to application (day 0) were considered as viable. The numbers of eggs and juvenile and adult *O. cynotis* specimens were counted and recorded both before (day 0) and after treatment (days 10 and 30) (Figure 1).

2.6. Scoring method
The degree of improvement was determined based on the scoring of clinical findings (such as erythema, ulceration, pruritus, pain, amount of discharge, and types of secretion) observed during pretreatment (day 0) and posttreatment (days 10 and 30) physical and otoscopic examinations. Clinical findings were scored between 0 and 3 depending

Figure 1. Adult *O. cynotis* mites and female with a visible egg.

on severity (0: absent, 1: mild, 2: moderate, and 3: severe) as reported previously (22–24).

2.7. Determining the efficacy percentage

The clinical efficacy percentage of the substances applied to the different groups was determined using the following formula after treatment (days 10 and 30) for each lifecycle stage (egg, juvenile, and adult) as determined previously (25).

Efficacy (%) = $100 \times (T_{bt} - T_{at}) / T_{bt}$

T_{bt} = Total number of mites before treatment

T_{at} = Total number of mites after treatment (days 10 and 30)

2.8. Statistical analysis

The groups were assessed statistically in terms of live mite numbers, clinical findings, clinical scores, parasitological findings, and efficiency percentages. One-way ANOVA testing was performed to determine the effect of different substances on treatment, with the results expressed as mean ± standard error. The clinical scoring of intergroup comparisons was performed using the Bonferroni test. Statistically, $P < 0.05$ was considered as significant.

3. Results

3.1. The number of mites and clinical findings

Live mite numbers were determined as the mean of the sum of both ears (Table). The difference between groups was not statistically significant ($P > 0.05$).

In G1, six of seven cases showed clinical improvement by day 10 (85%), while five of seven cases in G2 showed clinical improvement by day 10 (71%). In addition, five cases out of seven in G3 displayed clinical improvement by day 10 (71%), while all cases in G4 (100%) made a full clinical recovery by day 10. Clinical examinations performed on day 30 showed no changes in animals that exhibited clinical improvement by day 10, with no recurrence being observed in fully recovered cases. Clinical examination of cats treated in G1 and G4 on day 10 showed full recovery of the ear epithelia, while epithelial rashes were observed in two of the seven cats treated in G2 and in one of the seven cats treated in G3. As such, the ear epithelia healing rate was determined as 100% for G1 and G4, 85% for G3, and 71% for G2.

Table. Clinical scores and numbers of live mites (mean ± standard error).

Treatment group		Number of live mites[a]	Erythema	Ulceration	Pruritus	Pain	Secretion amount	Secretion type	Total
Day 0	G1	107.9 ± 53.4	0.7 ± 0.3	0.9 ± 0.3	0.2 ± 0.3	1.3 ± 0.3	2.3 ± 0.3	2.7 ± 0.2	8.1 ± 0.1
	G2	91.3 ± 39.7	0.7 ± 0.4	0.7 ± 0.4	2.0 ± 0.2	1.3 ± 0.3	2.0 ± 0.2	2.7 ± 0.2	9.4 ± 0.2
	G3	204.9 ± 128.8	1.0 ± 0.4	0.6 ± 0.4	2.3 ± 0.3	1.9 ± 0.4	2.1 ± 0.3	2.7 ± 0.2	10.6 ± 0.2
	G4	40.6 ± 17.4	0.1 ± 0.3	0.9 ± 0.4	1.9 ± 0.3	1.6 ± 0.2	2.0 ± 0.2	2.3 ± 0.2	8.8 ± 0.1
Day 10	G1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.3[b]
	G2	1.0 ± 0.7	0.4 ± 0.3	0.1 ± 0.1	0.4 ± 0.3	0.3 ± 0.2	0.4 ± 0.3	0.6 ± 0.4	2.2 ± 0.1[b]
	G3	1.0 ± 0.9	0.3 ± 0.2	0.0 ± 0.0	0.3 ± 0.3	0.1 ± 0.1	0.4 ± 0.3	0.9 ± 0.6	0.7 ± 0.1[b]
	G4	NO	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[b]
Day 30	G1	0.4 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[b]
	G2	1.0 ± 0.7	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.4	0.6 ± 0.4	1.5 ± 0.1[b]
	G3	1.0 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.1[b]
	G4	NO	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[b]

[a]No significant difference between groups ($P > 0.05$).
[b]Means are significantly different compared to day 0 ($P < 0.05$).
NO: No observed mites.

3.2. Clinical scoring findings

All the cats in the study were scored based on clinical findings (erythema, ulceration, pruritus, pain, secretion amount, and secretion type) before (day 0) and after (days 10 and 30) treatment, and the level of decrease in these scores was also determined as the mean of the sum of both ears (Table). Statistical assessment of intragroup differences for these clinical scores revealed that, on day 10 after treatment, the decrease in clinical scores was significant (P < 0.05), while the differences between G2 and G3 for erythema scores and the ulceration scores for all groups were not significant. On the other hand, no statistically significant difference was identified between the groups in terms of efficacy (P > 0.05).

3.3. Efficacy percentage findings

The efficacy of the substances was determined using the aforementioned formula and given as a percentage (EP). As such, when EP was evaluated on day 10 with regard to egg load, G4 was determined to have the highest efficacy (100%), followed by G2 (99.77%), G1 (99.63%), and G3 (99.28%). On day 30, G1 and G4 were similar in terms of efficacy (100%), followed by G3 (99.27%) and G2 (97.92%). According to the EP values, G1, G3, and G4 had similar efficacy (100%) on day 10 against juvenile *O. cynotis* specimens, while the lowest efficacy was observed for G2 (99.19%). On day 30, G1 and G4 were equal in terms of efficacy (100%) against juvenile *O. cynotis*, followed by G3 (99.85%) as the group with the second-highest efficacy and G2 (98.36%) as the group with the least efficacy. On day 10, G1 and G4 had the highest efficacy (100%) against adult *O. cynotis* specimens, followed by G3 (99.11%) and G2 (98.74%). On day 30, G4 exhibited the highest efficacy (100%) against adult *O. cynotis*, followed by G1 (99.77%)

and G3 (99.24%), while G2 (98.74%) had the least efficacy (Figure 2).

4. Discussion

This study was designed to compare efficacy of garlic oil (*Allium sativum* L.), marjoram oil (*Origanum majorana* L.), ozonated olive oil, and permethrin for the treatment of *O. cynotis* infestation in cats.

Many studies have been conducted on the treatment of *O. cynotis* infestation in cats with modern drugs such as permethrin (10,11,14,21,22,24–26). While ethnoveterinary uses of essential oils for treatment of ear mites of cats were mentioned in reviews (18), there is no experimental study conducted on this topic.

The efficacy of a licensed permethrin product (Oridermyl) for *O. cynotis* infestation in naturally infested cats was reported (22,24) and no live mites were observed otoscopically after treatment at day 10. In the current study, permethrin also presented a similar effect as reported previously.

In recent years, undesirable side effects associated with modern drugs have increased the popularity of drugs derived from natural sources and especially herbal treatments in the world (18).

An ethnoveterinary study performed by Lans et al. (18) stated the benefits of the medicinal plants of *A. sativum* L., *Thymus vulgaris* L., and *Origanum vulgare* for fleas and ear problems of cats and dogs. However, no study on the use of *A. sativum* L. and *O. majorana* L. oils against *O. cynotis* infestation exists. *Thymus vulgaris* L. and *Origanum vulgare* L., species of thyme containing similar active substances (carvacrol, thymol, terpinen-4-ol) as *O. majorana* L., were used for ear infections, and no associated toxic effects were
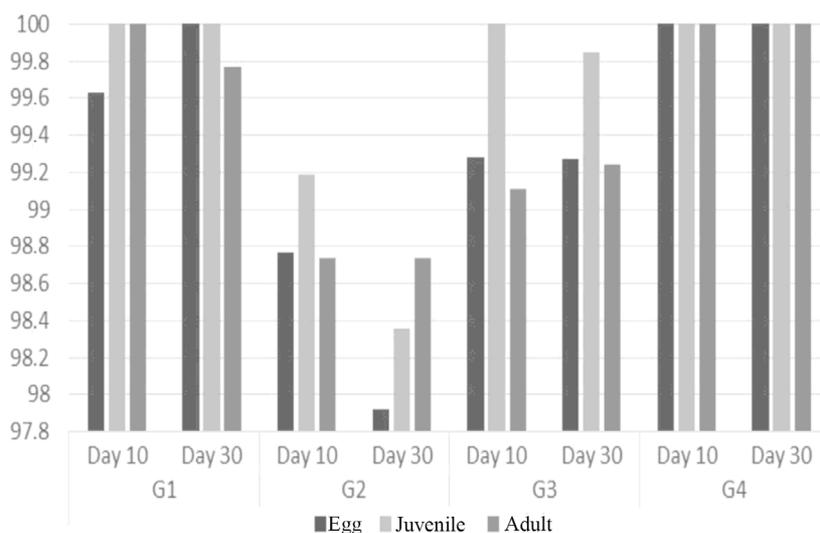
Figure 2. Clinical efficacy (%) of groups (days 10–30).

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