Original Article

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.1061271
J Health Sci Med 2022; 5(2): 558-563

# Comparison of the in vitro *Demodex folliculorum* killing activity of azelaic acid and permethrin

**Ayşenur Botsali[1], Aslan Yurekli[2]**

[1]University of Health Sciences, Gülhane Training and Research Hospital, Dermatology Department, Ankara, Turkey
[2]Kuşadası State Hospital, Dermatology Department, Aydın, Turkey

**Cite this article as**: Botsali A, Yürekli A. Comparison of the in vitro *Demodex folliculorum* killing activity of azelaic acid and permethrin. J Health Sci Med 2022; 5(2): 558-563.

## ABSTRACT

**Aim**: *Demodex* parasites have been linked to dermatological disorders, especially rosacea, without a proven mechanism. Moreover, some anti-*Demodex* agents demonstrate a dual therapeutic effect related to a direct effect on the skin disorder along with a decreased number of *Demodex* mites. Despite being considered a first-line treatment approach for rosacea, azelaic acid's efficacy against *Demodex* mites has not been investigated. In the current study, mites were continuously observed after exposure to the test agents to evaluate the potential anti-*Demodex* efficacy of azelaic acid. The efficacy of azelaic acid was compared to that of a positive control agent (permethrin).

**Material and Method**: The wastes of diagnostic standardized skin surface biopsy samples of rosacea patients were collected for the trial. To four active treatment groups were administered 10% azelaic acid, 20% azelaic acid, 30% azelaic acid, and 5% permethrin. In addition, there was a control group, and 20 *Demodex* mites were included in each of the five groups. The authors conducted the real-time observation of the study groups through a digital microscope. The survival times of the mites were recorded and compared between the groups.

**Results**: The mean survival time was 12.2±1.5 minutes in the 5% permethrin group. The mean survival times in the 10%, 20%, and 30% azelaic acid groups were 15.8±1.6, 14±1.5, and 12±1.2 minutes, respectively. The differences between the four active treatment groups did not reach statistical significance ($p>0.05$).

**Discussion**: The present study's results revealed that all three concentrations of azelaic acid had anti-*Demodex* efficacy comparable to that of 5% permethrin.

**Keywords**: *Demodex*, rosacea, topical treatment

## INTRODUCTION

*Demodex* mites reside in hair follicles and sebaceous glands and survive by the ingestion of keratin and sebaceous secretions. The presence of *Demodex* mites in healthy humans is common. However, they are linked to severe infestations that result in mortality in animals (1). Thus, in contrast to the established pathogenic potential in animals, they are mostly accepted as commensals in humans (2). Among the mites that settle on the skin, such as Sarcoptes scabiei hominis, *Cimex lectularius*, and *Dermatophagoides pteronyssinus*/*farinae*, *Demodex* mites are less immunogenic and harmless, rarely causing immunological or allergic reactions (3).

In humans, *Demodex* mites localized at the mother's nipple pass from mother to infant shortly after birth (4). *Demodex* mites contain lipase enzymes and they tend to settle in seborrheic areas, especially the facial skin. Activation of the sebaceous glands in adolescence creates a relatively favorable microenvironment for the development of *Demodex* mites, and an increase in *Demodex* density is observed during this period. While the incidence is 13% in the population between 3 and 15 years old, it reaches 95% in those over 70 (4, 5).

*Demodex* mites have been linked to various ophthalmological and dermatological disorders (4, 6-8). Although a causal explanation has yet to be established, several studies have demonstrated that *Demodex* density was substantially increased in rosacea, perioral dermatitis, and folliculitis patients compared to age- and sex-matched control groups (8-11). *Demodex* mites were hypothesized to cause permanent microabrasions within the skin of rosacea patients. Accordingly, the deterioration of the skin barrier might contribute to cutaneous hypersensitivity. Hence, a reduction in *Demodex* density might relieve the symptoms and provide better disease control in rosacea patients.

**Corresponding Author:** Ayşenur Botsalı, abotsali@hotmail.com
**Received:** 13.12.2021 **Accepted:** 09.02.2022

Although it is possible to detect *Demodex* mites through histopathological examination of punch biopsy samples, the ideal diagnostic method for cutaneous demodicosis is the standardized superficial skin biopsy (SSSB) (12). The skin surface biopsy, which was first described in 1971, was revised by Forton and Says in 1993 and later renamed the SSSB (14, 15). For this practical, noninvasive technique, a 1 cm2 square is drawn on a slide. After cyanoacrylate is dripped onto this site, the slide is attached to the target sample collection area and kept in the same region for 60 seconds. After that, the slide is gently removed and the sample is examined by direct microscopy. This method can detect large numbers of *Demodex* mites and the movement of these mites can be easily observed due to their relatively large size. Although a dermatologist can easily perform the technique even in a first-line hospital setting, studies on acaricidal agents' in vitro anti-*Demodex* effect are very limited. In the study by Kligman et al., in which double-sequence standardized skin surface biopsy techniques were described, the average survival time of *Demodex* mites obtained by this method was 3 hours in olive oil. In comparison, it was less than 2 hours in mineral oil (10). This suggests that a follow-up period of approximately three hours would be sufficient to evaluate the efficacy of any treatment agent on *Demodex*.

A limited number of studies on aromatic oils' in vitro anti-*Demodex* activity have been published (16-22). In these studies, eyelash samples were exposed to the treatment agents. However, it was stated that parasites embedded in highly keratinized hair samples would be protected against drug penetration. Recently, our research group conducted an in vitro experiment on the wastes of SSSB samples of rosacea patients (23). We compared the anti-*Demodex* efficacy of tea tree oil to that of permethrin (positive control) and immersion oil (negative control). By using this approach, we detected a dose-related response pattern for tea tree oil. The survival time of the negative control group was 196 minutes, which was compatible with the available data (23).

The data for *Demodex* treatment approaches are quite limited. An in vitro study conducted in 1981 revealed that metronidazole, which is considered in the forefront of anti-*Demodex* treatments, did not alter the survival of *Demodex* mites even at high doses such as 1 mg/kg (24). Thus, the efficacy of metronidazole has been associated with an independent anti-inflammatory action rather than a direct acaricidal effect (24). Recently, it has been suggested that the successful treatment results obtained with ivermectin in rosacea cases may be related to the combination of a decrease in *Demodex* density and an anti-inflammatory effect (25). The intertwining of different mechanisms makes it difficult to determine whether the positive result obtained is due to the acaricidal effect or the direct effect of the drug on the underlying dermatological disease.

Conversely, accepted treatment alternatives for rosacea may also have direct anti-*Demodex* efficacy. Azelaic acid (1,7-heptanedicarboxylic acid) is a naturally occurring saturated dicarboxylic acid (26, 27). The anti-inflammatory, antibacterial, and antikeratinizing effects of azelaic acid have been described. It can inhibit tyrosinase, which is involved in the production of melanin, and also 5α-reductase, which is related to androgenetic alopecia (26, 27). It has been used in various formulations to treat rosacea, acne, and melasma. Azelaic acid 15% gel has been approved by the US Food and Drug Administration for the topical treatment of inflammatory papules and pustules of mild to moderate rosacea (28). There have been no reports on the anti-*Demodex* efficacy of this agent. The aim in the present study was to investigate azelaic acid's in vitro *Demodex* killing activity by using our recently published technique.

## MATERIAL AND METHOD

The University of Health Sciences Gülhane Scientific Researches Ethics Committee approved the study (Date: 06.01.2022, Decision No: 2022/10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

### Materials

The study agents, topical permethrin 5% solution and azelaic acid, were provided by the company Jeomed (Istanbul, Turkey). Azelaic acid in 10%, 20%, and 30% forms was prepared and used during the experiments.

### Patients

The wastes of rosacea patients' diagnostic SSSB samples from two different outpatient clinics were used for the experiments. Specimens demonstrating live *Demodex* mites were selected. The authors excluded the *Demodex* mites in their early life cycle due to their increased susceptibility to therapeutic agents. Considering that parasite viability may differ among specimens with different inclusion time points, the authors evaluated the presence of *Demodex* mites during an average duration of 30-45 seconds in all of the experiments. Afterward, the most active *Demodex* mite was determined as the target and the treatment agents were immediately applied. The study agents were directly injected onto the *Demodex* mites. The movements of the mites were continuously observed via the screen of a digital microscope.

### Experimental Design

The study included five experimental groups. Twenty *Demodex* mites were included in each group and 10% azelaic acid, 20% azelaic acid, 30% azelaic acid, or 5% permethrin was applied. In the control group, the movements of the mites were observed after dripping only immersion oil.

Although immersion oil is essential for a detailed examination of mite movements and morphological features when examining SSSB samples, the authors, in their previous observations, found that immersion oil might also reduce the penetration of treatment agents. For this reason, all of the samples included in the study were first scanned at 10× and 40× magnification without dripping immersion oil and roughly evaluated for the presence of *Demodex* mites. The eligible SSSB samples were exposed to treatment agents prepared in either immersion oil or the oily solutions of active treatment agents to enable clear field monitoring at large magnification. The samples were evaluated with a digital microscope (Bresser Optics, Digital LCD Microscope, Germany) with 40× optical zoom to 1600× digital zoom magnification (**Figure 1**). The authors determined the most mobile *Demodex* mite the target in each sample and the monitoring area was fixed to this region. Vitality was assessed by our recently defined method, i.e., the continuous observation of this site on the digital screen for the movement of *Demodex* body and legs, up to a maximum of 240 minutes (23). The authors defined the survival time (ST) of *Demodex* mites as the interval (min) between the first exposure of the mites to the working solution and when their motility ceased. Cessation of movement was defined as the complete absence of trunk and limb movements for 60 seconds. The authors did not apply any manipulation to the mites during the experiments. The mean ST was compared between the five study groups to evaluate the potential in vitro *Demodex* killing activities. Furthermore, observations on the morphological appearance of the *Demodex* mites were recorded and assessed during this period.

### Statistical Analysis

The exposure of live *Demodex* mites to each study solution was repeated twenty times on independent occasions. Statistical analyses were performed using IBM SPSS for Windows, Version 22.0. Numerical variables were shown as mean±SD. Differences between the groups were evaluated by a two-tailed t-test. A p-value <.05 was considered significant in all comparisons.

## RESULTS

All of the mites included in the study were *Demodex folliculorum*. The study did not include *Demodex* brevis mites, which were rarely observed in SSSB samples. All 80 mites exposed to the active treatment solutions except those in the control group (immersion oil) were completely free of movement within the first 20 minutes of exposure (min-max: 10-18 minutes). Mean ST was 15.85±1.6, 14.05±1.5, and 12±1.2 minutes in the 10%, 20%, and 30% azelaic acid groups, respectively. The differences between the three groups did not reach statistical significance (p>0.05) (**Table 1**). The mean ST was 12.2±1.5 minutes in the 5% permethrin group. In the comparative evaluation of the permethrin group with the azelaic acid groups, no difference was observed in terms of ST (p>0.05) (**Table 1**). The mean ST of the control group was 197±23.6 minutes.

**Figure 1.** The digital microscope enabling the continuous observation of *Demodex* movements

**Table 1.** The mean±SD survival time in the five experimental groups - p values

| | | 5% Permethrin | 10% AA | 20% AA | 30% AA | Negative Control |
|---|---|---|---|---|---|---|
| | | **12.2±1.5** | **15.85±1.6** | **14.05±1.5** | **12±1.2** | **197±23.6** |
| 5% Permethrin | 12.2±1.5 | - | 0.735 | 0.691 | 0.168 | 0.000 |
| 10% AA | 15.85±1.6 | 0.735 | - | 0.449 | 0.067 | 0.000 |
| 20% AA | 14.05±1.5 | 0.691 | 0.449 | - | 0.343 | 0.000 |
| 30% AA | 12±1.2 | 0.168 | 0.067 | 0.343 | - | 0.000 |
| Negative Control | 197±23.6 | 0.000 | 0.000 | 0.000 | 0.000 | - |

AA: Azelaic acid

Morphological degeneration findings were detected simultaneously with the cessation of movement in all of the mites in the permethrin group. Morphological degeneration consisted of trunk shrinkage and either blunting or a complete loss of claw and nail structures within the extremities of the *Demodex* mites (**Figure 2**). However, the protrusions depicting extremities were still visible on the lateral sites of the mites during later examinations. As an exceptional finding, fragmentation of mites was observed. These mites were also screened after the cessation of movement and the loss of lateral protrusions was a late finding (**Figure 2**). These differences between the early- and late-stage observations suggested the contraction was related to paralysis of the mites.

None of the mites in the azelaic acid or control groups showed any alteration in body integrity or signs of morphological degeneration. The parasites had a linear appearance related to the eversion of the extremities and the loss of lateral protruding structures (**Figure 3**).

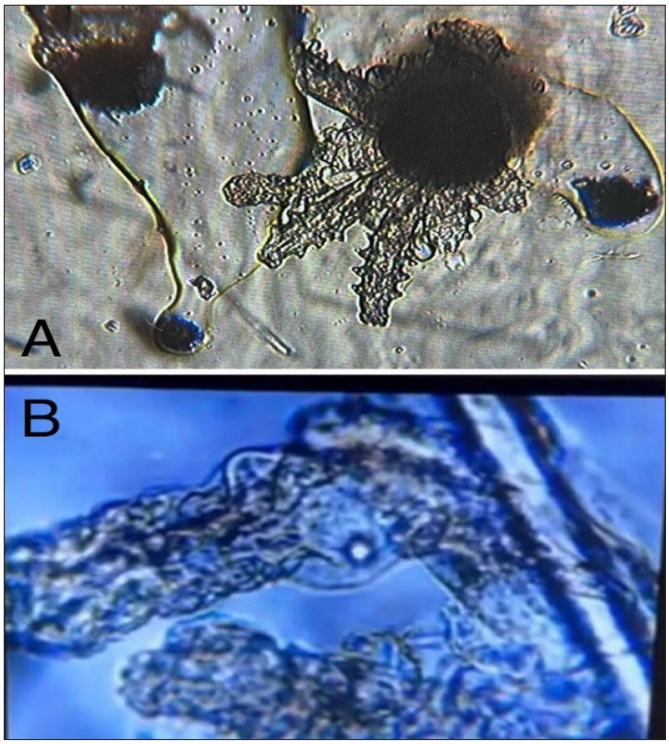


**Figure 2.** *Demodex* mites in the permethrin group. A) Trunk shrinkage and the loss of delicate features within the limbs. However, the lateral protrusions are prominent, indicating contraction. B) Fragmentation of the trunk and loss of the prominent lateral protrusions


**Figure 3:** *Demodex* mites in the azelaic acid group. The eversion of the lateral structures led to a linear appearance concurrent with the end of movement.

## DISCUSSION

In the current study, by using our recently developed technique, we found that different azelaic acid concentrations had an in vitro anti-*Demodex* efficacy comparable to that of permethrin 5%. Although numerical differences were detected in ST between the different azelaic acid concentrations, these differences did not reach statistical significance. All concentrations of azelaic acid between 10% and 30% provided efficacy similar to that of permethrin 5%. All the active treatment agents had a significant effect on the ST of *Demodex* mites compared to the negative control group.

Although acaricidal agents are frequently incorporated in the management of rosacea with successful treatment outcomes, there is no consensus yet on the optimal dose and treatment duration of these regimens or long-term follow-up results (9). Azelaic acid is a versatile, effective dermatological treatment agent used to treat different cutaneous disorders like melasma, acne, and rosacea related to different action mechanisms (27). *Demodex* mites are sensitive to alterations within their microenvironment. As a typical example, systemic isotretinoin treatment can dramatically decrease *Demodex* density related to the inhibition of sebum production (1). Thus, in clinical practice, several agents can provide decreases in *Demodex* density related to either a direct effect on the mites or an indirect effect related to other changes within the skin. However, instead of an indirect effect related to microenvironmental changes, the results of our study revealed that azelaic acid might have an effect profile similar to that of permethrin on *Demodex* mites, in addition to its well-known antibacterial effects.

In another study, we recently compared the in vitro *Demodex* killing activity of tea tree oil to that of permethrin (23). During the experiments, only the permethrin group had notable findings suggesting morphological degeneration, even fragmentation (23). None of the samples in the tea tree oil groups demonstrated these findings. Thus, the morphological features were also examined in detail in the present study. Upon morphological evaluation of *Demodex* mites, degeneration findings were observed only in the permethrin group. These findings differed between the early stage and late stage. On the other hand, the

morphological features of the azelaic acid groups were identical to those of the negative control group. From this perspective, azelaic acid's *Demodex* killing potential is not identical to that of permethrin and the preservation of the mites' integral structure may prevent the exacerbation of patient-related symptoms.

A common scenario clinicians experience after introducing acaricidal treatment in patients with high *Demodex* density is increased irritation and erythema. The exacerbation of the symptoms may be related to a hypersensitivity reaction to the dead *Demodex* mites. Although typically the intact mites do not evoke an inflammatory response, the fragmentation and degeneration of the mites might cause this exaggerated response (2). This phenomenon is frequently observed with permethrin, a major cause of treatment incompatibilities. Hence, shorter-duration topical applications are often used at the initial stages of permethrin treatment, and the applications are gradually increased. The intense morphological degeneration recorded in the permethrin group may contribute to this situation. The acaricidal effect provided by azelaic acid without this morphological degeneration can represent an advantage to prevent these exacerbations. This prediction needs to be supported by clinical observations and studies on this subject. However, despite being considered a first-line treatment approach for rosacea, azelaic acid can have an irritative potential on the skin independent of *Demodex* mites. Thus, similar to permethrin, rosacea patients with increased *Demodex* mites should be treated with a gradually increasing treatment scheme of azelaic acid.

The findings of the present study are limited to in vitro experiments and do not entirely reflect the efficacy of these agents in clinical practice. Another study limitation is that a dose-dependent response pattern could not be demonstrated for azelaic acid and all three study concentrations had similar effects. The preferences for selecting the drug concentrations were determined according to routine clinical practice. Azelaic acid concentrations below 10% were not included in the study due to the use of 15% azelaic acid in rosacea.

## CONCLUSION

In addition to the versatile efficacy of azelaic acid for dermatological diseases, the present study's findings revealed an acaricidal effect similar to that of permethrin 5% on *Demodex folliculorum*. Azelaic acid is a first-line treatment for rosacea. Considering the relationship between rosacea and *Demodex* mites, we think that azelaic acid is also an acceptable agent in rosacea patients with high *Demodex* density and may eliminate the need for additional acaricidal treatments. Azelaic acid can also minimize the possibility of hypersensitivity reactions related to the degeneration of mites. This advantage of azelaic acid may make it preferable to permethrin for cutaneous demodicosis, considering the altered skin barrier of these patients.

## ETHICAL DECLARATIONS

**Ethics Committee Approval**: The University of Health Sciences Gülhane Scientific Researches Ethics Committee approved the study (Date: 06.01.2022, Decision No: 2022/10).

**Informed Consent**: All patients signed the free and informed consent form.

**Referee Evaluation Process**: Externally peer-reviewed.

**Conflict of Interest Statement**: The authors have no conflicts of interest to declare.

**Financial Disclosure**: The authors declared that this study has received no financial support.

**Author Contributions**: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

**Acknowledgment**: The authors would like to thank Jeomed Company (Turkey) for providing the study solutions.

## REFERENCES

1. Elston CA, Elston DM. *Demodex* mites. Clin Dermatol 2014; 32: 739-43.
2. Lacey N, Raghallaigh SN, Powell FC. *Demodex* mites-commensals, parasites or mutualistic organisms? Dermatology 2011; 222: 128.
3. Chen W, Plewig G. Human demodicosis: revisit and a proposed classification. Brit J Dermatol 2014; 170: 1219-25.
4. Czepita D, Kuźna-Grygiel W, Kosik-Bogacka D. Investigations on the occurrence as well as the role of *Demodex* follicuforum and *Demodex* brevis in the pathogenesis of blepharitis. Klinika Oczna 2005; 107: 80-2.
5. Ivy SP, Mackall CL, Gore L, Gress RE, Hartley AH. Demodicidosis in childhood acute lymphoblastic leukemia: an opportunistic infection occurring with immunosuppression. J Pediatr 1995; 127: 751-4.
6. Aktaş Karabay E, Aksu Çerman A. *Demodex folliculorum* infestations in common facial dermatoses: acne vulgaris, rosacea, seborrheic dermatitis. An Bras Dermatol 2020; 95: 187-93.
7. Chang Y-S, Huang Y-C. Role of *Demodex* mite infestation in rosacea: A systematic review and meta-analysis. J Am Acad Dermatol 2017; 77: 441-7.e6.
8. Forton FMN. The pathogenic role of *Demodex* mites in rosacea: a potential therapeutic target already in erythematotelangiectatic rosacea? Dermatol and Ther 2020: 1-25.
9. Jacob S, VanDaele MA, Brown JN. Treatment of *Demodex*-associated inflammatory skin conditions: a systematic review. Dermatol Ther 2019; 32: e13103.
10. Kligman AM, Christensen MS. *Demodex folliculorum*: requirements for understanding its role in human skin disease. J Invest Dermatol 2011; 131: 8.

11. Lacey N, Russell-Hallinan A, Zouboulis CC, Powell FC. *Demodex* mites modulate sebocyte immune reaction: possible role in the pathogenesis of rosacea. Brit J Dermatol. 2018; 179: 420-30.
12. Aşkın Ü, Seçkin D. Comparison of the two techniques for measurement of the density of *Demodex folliculorum*: standardized skin surface biopsy and direct microscopic examination. Brit J Dermatol 2010; 162: 1124-6.
13. Aytekin S, Göktay F, Yaşar Ş, Gizlenti S. Tips and tricks on *Demodex* density examination by standardized skin surface biopsy. J Eur Acad Dermatol Venereol 2016; 30: e126-e8.
14. Forton F, Seys B. Density of *Demodex folliculorum* in rosacea: a case-control study using standardized skin-surface biopsy. Brit J Dermatol 1993; 128: 650-9.
15. Marks R, Dawber RPR. Skin surface biopsy: an improved technique for the examination of the horny layer. Brit J Dermatol 1971; 84: 117-23.
16. Du J, Gao R, Zhao J. The Effect of Volatile Oil from Chinese Mugwort Leaf on Human Demodecid Mites In Vitro. Acta Parasitol 2021; 66: 615-22.
17. Gao YY, Di Pascuale MA, Li W, et al. In vitro and in vivo killing of ocular *Demodex* by tea tree oil. Brit J Ophthalmol 2005; 89: 1468-73.
18. Jixin L, Chaopin L, Yanhon S, et al. The effect of the volatile oil of Manchurian Wildginger on the killing of *Demodex* in vitro. J Trop Dis Parasitol 2014; 12: 21.
19. Lam NSK, Long XX, Li X, Yang L, Griffin RC, Doery JCG. Comparison of the efficacy of tea tree (Melaleuca alternifolia) oil with other current pharmacological management in human demodicosis: a systematic review. Parasitol 2020; 147: 1587-613.
20. Liu JX, Sun YH, Li CP. Volatile oils of Chinese crude medicines exhibit antiparasitic activity against human *Demodex* with no adverse effects in vivo. Exp Therap Med 2015; 9: 1304-8.
21. Oseka MK, Sedzikowska A. Effect of essential oils on the survival rate of *Demodex* spp. Invest phthalmol Vis Sci 2014; 55: 6252-.
22. Song B, Liu J, Liu T. Effect of galangal essential oil on killing human *Demodex* in vitro. J Path Biol 2010; 5: 155-60.
23. Yurekli A, Botsali A. The comparative in vitro killing activity of tea tree oil versus permethrin on *Demodex folliculorum* of rosacea patients. J Cosmet Dermatol 2022; doi: 10.1111/jocd.14701.
24. Persi A, Rebora A. Metronidazole and *Demodex folliculorum*. Acta Derm Venereol 1981; 61: 182-3.
25. Abokwidir M, Fleischer AB. An emerging treatment: topical ivermectin for papulopustular rosacea. J Dermatol Treat 2015; 26: 379-80.
26. Schulte BC, Wu W, Rosen T. Azelaic acid: evidence-based update on mechanism of action and clinical application. J Drugs Dermatol 2015; 14: 964-8.
27. Searle T, Ali FR, Al-Niaimi F. The versatility of azelaic acid in dermatology. J Dermatol Treat 2020: 1-11.
28. Gollnick H, Layton A. Azelaic acid 15% gel in the treatment of rosacea. Exp Opin Pharmacother. 2008; 9: 2699-706.