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Comparison of sensitization antigens of the brown-banded cockroach (*Supella longipalpa*) and German cockroach (*Blattella germanica*) in the animal model

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Abstract

Cockroaches are one of the most important causes of asthma and allergies in residential areas. Sensitization to cockroach allergens is an established risk factor for asthma among populations. In this study, cockroach bodies and feces from the two species were separately dried, homogenized, and centrifuged after one day in phosphate-buffered saline (PBS). The protein concentration of the samples was then determined using the Bradford method. The supernatant was prepared for injection into 20 mice, and after four weeks of subcutaneous injection, an ELISA test was used to determine the antibody titer against the injected antigen. After the overdoes (OD) samples were detected, an SDS-PAGE test was performed to determine the peptides' molecular band and molecular weight. Lastly, a Western blot test with anti-mouse IgE conjugated HRP (Horseradish Peroxidase) for IgE in mouse serum was prepared. A comparison of the cockroach OD with the rest showed that more antibodies, both for feces and for the body, were produced in the mouse serum than in other cockroaches. A Western blot test also showed that the body of this cockroach produced IgE antibodies in the serum of mice. The molecular weights of body and feces samples of German cockroaches by SDS-PAGE were 85, 75, and 100 kDa, respectively, and 75, 35, 60, and 60 kDa, respectively, for the body and feces of the brown-banded cockroach. The weight of IgE protein bands (antibody) produced in mouse serum by western blot varied from 60 to 220 kDa. The upper titer of the whole body and the cockroach feces of *Supella longipalpa* were observed in comparison with the other cockroaches, as well as the IgE antibody band of the Supella whole body was observed, which indicated the risk of allergy to this cockroach.

1. Introduction

The order Blattodea is one of the 31 orders of insects that exist in almost all parts of the world. Cockroaches are insects that date back 400 million years. So far, more than 4,500 species of cockroach have been identified (Kambhampati 1995). The most common cockroaches worldwide

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are the American (*Periplaneta americana*), the German (*Blattella germanica*), the Oriental (*Blatta orientalis*), and the brown-banded (*Supella longipalpa*) cockroach (Nasirian and Salehzadeh 2019). In addition to the physical transmission of some pathogens, cockroaches are one of the most important causes of asthma and allergies in residential areas. Sensitization to cockroach allergens is an established risk factor for asthma among inner-city populations (Gelber et al. 1993, Eggleston et al. 1998).

Saliva, feces, and all parts of the cockroach body that are spread in the house are allergenic and can cause shortness of breath, cough, respiratory inflammation, and asthma (Gould and Deay 1940, Leishangthem et al. 2021). The abundance of cockroaches, including their saliva, feces ,and entire body, is a cistern of allergens and can cause allergies and asthma in people, particularly children. Approximately 40%-60% of asthma patients in urban and inner-city regions possess IgE antibodies to cockroach allergens (Hashemi-Aghdam and Oshaghi 2015). German cockroaches are common species and important sources of household allergens (Hubner-Campos et al. 2013, Carlson, Rabito et al. 2017). In the late 1990s, the national cooperative inner-city asthma study (NCICAS) proved that urban children with asthma who were both susceptible and exposed to high levels of cocroach allergen at home had increased asthma morbidity (Esty et al. 2019). The brown stripe cockroach (*Supella longipalpa*) is a smaller species of cockroach in the world and has been introduced as a mechanical carrier of various pathogens and allergen sources (Adler 1985).

Supella longipalpa (Blattaria:Blattellidae) is an emerging urban pest in Iran and has recently infested buildings and hospitals over a wide area in Iran (Sedghiany 2000, Nasirian and Salehzadeh 2019). Like *Blattella germanica* (Nasirian 2010), *Supella longipalpa* carries a variety of microorganisms as a vector for pathogenic bacteria in an urban environment, including Iran, as reported by Vazirianzadeh et al. (Vazirianzadeh, et al. 2014). This cockroach has a global spread and is able to transmit many pathogens, such as bacteria, viruses, fungi, protozoa, and parasite eggs, to humans mechanically or through its gastrointestinal tract. *Supella longipalpa* is a source of allergen (Gould and Deay 1940, Gore and Schal 2007, Rodriques 2021). *Supella longipalpa* lives in hot and dry environments and may be found in all areas of residence, such as bedrooms and living rooms. The lifespan of an adult is thirteen to forty-five weeks, and each female produces 600 offspring each year (Cochran 1999, Sedghiany 2000, Nasirian 2016). No information is available about the possibility of allergens in the *Supella longipalpa* cockroach, so this study is an introduction to investigating the possibility of allergies and sensitization by this cockroach. Since the occurrence of any allergy first requires the production of antibodies, it is necessary to first examine the potential for IgE antibody production by this cockroach in the animal model.

2. Material and methods

2.1 Preparation of the cockroach body and feces extract

The process of preparing cockroach antigens was performed according to the instructions of Thangam Sudha and colleagues (Thangam Sudha et al. 2007). First, two colonies of the two cockroach species (*B. germanica* and *S. longipalpa*) were reared separately in sterile buccal incubators at the School of Public Health, Tehran University of Medical Science cockroach insectarium. After one month and a generation increase, 30 cockroaches were isolated from each species and transferred to sterile plates. After three days, the cockroaches whose intestines had been emptied of feces were transferred to the oven for drying for 24 hours at 39 ° C. After complete drying, the cockroach bodies were entirely pulverized with a mortar (Thangam Sudha et al. 2007).

Simultaneously, feces of both species were collected from the floors of the breeding buccals and pulverized by manual homogenization. Powder from feces and whole bodies was

dissolved separately in a ratio of 1:25 in phosphate-buffered saline (PBS) 0.01 M (pH 7.4). After standing at 4 ° C for one day, a shaker stirred the solution for 8 hours. After this time, the solution was centrifuged at 13,000 rpm for 1 hour, and the resulting supernatant was filtered and dialyzed in PBS twice. Finally, the extract was filtered through a 0.22 µm membrane under sterile conditions. Protein concentration for all extracts was determined using Bradford's method (Thangam Sudha et al. 2007).

2.2 Animals

Twenty four-month-old male BALB/c mice weighing between 26-32 g were procured from the School of Public Health at Tehran University of Medical Science. All animals were housed under controlled temperature (22 ℃) and humidity (45%). The animals were maintained in controlled rooms with 12 h light cycles and had access to food and water. They were divided into two groups (n = 10). Ten mice were isolated for feces extract injection and 10 for whole body extract injection. Of the 10 feces antigen injections, 5 mice received *B. germanica*, and 5 received *S. longipalpa*. Separation of mice for injection of whole body extract was performed as described above. The Ethical Committee of Tehran University of Medical Science approved the study procedures. The ethical code assigned for this study was 9311263003.

2.3 Sensitization and injection of antigens into mice

Four injections were performed. The first injection, 100 µl of *B. germanica* body extract solution, was poured into an Eppendorf tube and mixed with 100 µl of Freund's complete adjuvant (to stimulate the immune system of mice). The material inside the Eppendorf was drawn and emptied several times using a 1 ml syringe to mix thoroughly. Then, the solution was drawn with an insulin syringe, and 200 microliters were injected subcutaneously into the area under the neck skin of five mice. Injections of the whole-body extract solution of *S.longipalpa* and the feces antigen solution of *S.longipalpa* were were done in the same way. After 2 weeks, the second injection, which played the role of Booster, was done similarly to the first injection, but instead of Freund's complete adjuvant, incomplete Freund's adjuvant was used. The third and fourth injections were performed in the same way. The concentration of injected proteins was 8.5 and 5.5 mg/ml for the body and feces of the German cockroach and 5.5 and 1.5 mg/ml for the body and feces of the brown-banded cockroach, respectively.

2.4 Serum isolation

Two weeks after the fourth injection, the mice were first anesthetized with a combination of xylazine and ketamine. The blood was then exsanguinated by cardiac puncture, and the collected blood was immediately centrifuged at 5,000 rpm for 10 minutes. Next, the supernatant was pooled and transferred to a new Eppendorf tube. In which the pooled serum samples were prepared and stored at −20° for immunoblotting.

2.5 Enzyme-linked immunosorbent assay (ELISA)

Mouse sera were quantitated for specific IgE to cockroach antigens using ELISA. In short, mouse serum (1:10 v/v) was incubated at 4 ℃ overnight on a microtiter plate covered with cockroach protein (1 µg/100 µl per well) in carbonate buffer, pH 9.6. The plate was probed with 1:1000 v/v diluted anti-mouse-IgE peroxidase (Sigma) after being cleaned with PBST (PBS containing 0.2% Tween 20) and PBS. In an ELISA reader, the hue produced with o-phenylene diamine was read at 492 nm (Karsonova et al. 2020).

2.6 ELISA inhibition

The effectiveness of the extract was evaluated using a specific IgE inhibition test. The microtiter plate was covered with 3% defatted milk and coated with crude cockroach protein (1 µg per well). Gradually increasing quantities of cockroach extract (0.1, 1, 10, 100, and 1000 ng) as an inhibitor were added to a pre-mixture of 1:10 diluted cockroach hypersensitive pooled

patient sera in a 100 µl volume. The following procedures followed those for ELISA.

2.7 SDS-PAGE of the Cockroach body and feces extract solution

The appropriate number of samples was poured into the Eppendorf tube and diluted 1:1 to 1:3 with the sample buffer. It was then placed in boiling water for 2 to 10 minutes. The cockroach extract solution was introduced to SDS-PAGE on 1% resolving gel; 5 µl of each extract solution was loaded into separate wells and electrophoresed at 100-120 V. The wells were named in order, and their direction was determined. A marker was used in a separate well to determine the molecular weight and the resolving gel was stained with Coomassie Brilliant Blue R-250 (CBBR).

2.8 Immunoblotting (Western blot)

SDS-PAGE separated protein was electrophoretically transferred onto a nitrocellulose membrane in Tris-glycine buffer containing methanol and probed with mice sera (pooled/individual). After blotting, the membrane was cut into strips, blocked with 3 % defatted milk, incubated with 1:10 v/v diluted cockroach mice sera, and shaken in a shaker for one hour. After positive and negative Control serum was considered, the strips were washed two or three times with Tris-buffered saline (TBS) and then probed with 1:500 v/v anti-Mouse IgE conjugated with Peroxidase (Sigma, Munich. Germany) antibody at 37 °C for 1 hours. The anti-Mouse IgE conjugated with Peroxidase solution was poured on the strips, which then were placed on the shaker for an hour and a half. They were then washed three times with TBS for 10 minutes each time. The color appeared after about 5 to 10 minutes, and IgE reactive bands were visualized with diaminobenzidine and hydrogen peroxide. Bound mouse IgE was detected with 1:5,000 goat anti-mouse IgE Horseradish peroxidase (Koma Co., Seoul, Korea).

3. Results and discussion

The Bradford method was used to measure the total protein concentration of allergen solutions in cockroach bodies and feces (Table 1). The body protein and feces concentrations of the two cockroach species were determined for the injection dose of the mice.

Table 1: Total protein concentration of body samples and feces of cockroach species.

Sample	Concentration (mg/ml)
German Cockroach body	5/5
German Cockroach feces	8/5
Supella Cockroach body	5/5
Supella Cockroach feces	1/5

The amount of light absorption of the OD samples obtained from the bodies and feces of two species of cockroaches (Fig. 1) in the ELISA test showed that the amount of light absorption IgE of the samples obtained from the body and feces samples are different, the amount of light absorption from the body of *Supella longipalpa* was more than the rest.

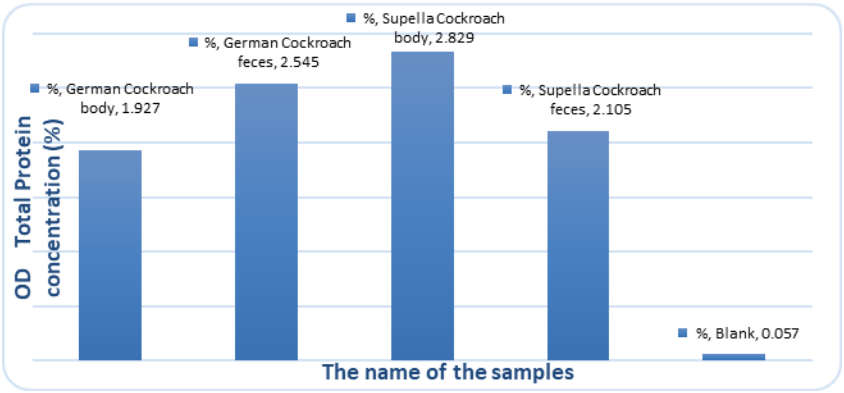

Figure 1: OD measured with ELISA (body samples and feces of cockroach species).

The results of the SDS-PAGE test showed that the samples obtained from the bodies of both species of cockroaches have several bands. While the bands of cockroach feces specimens had fewer bands, showing the amount of protein and peptides in feces is very low.

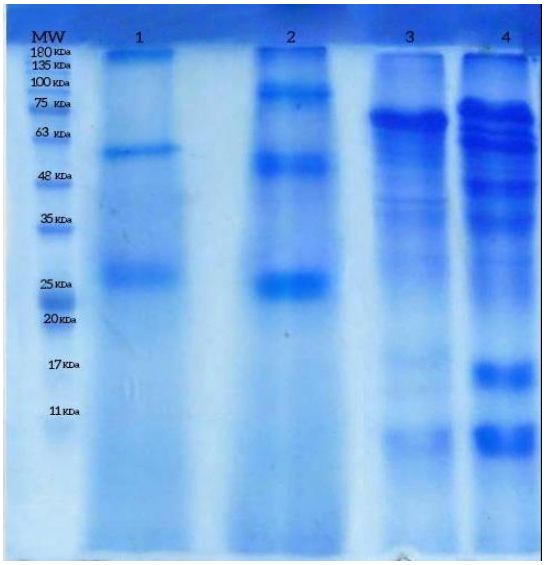

Figure 2: The stained gel related to the body and samples of both species, using 10% gel, from left to right: (1) Ledder Marker (MV) supella Longipalpa feces, (2)German cockroach feces, (3)Supella cockroach body, and (4) German cockroach body

Table 2: Molecular weight of body samples and cockroach feces in kDa by SDS-PAGE.

Sample	Molecular weight(kDa)
German Cockroach body	85-75
German Cockroach feces	100-85
Supella Cockroach body	75-35-60
Supella Cockroach feces	63

Western blot analysis revealed that the IgE antibody used identified two antibodies derived from the German cockroach body antigen, one of which was heavy. In the feces of this species, two peptide or protein bands with anti-IgE were shown. This indicates the presence and production of IgE antibodies resulting from the stimulation of the body antigen and German cockroach feces in the immune system of mice. For the brown-banded cockroach, an IgE antibody band corresponding to the cockroach body was shown, but no band was shown for the feces.

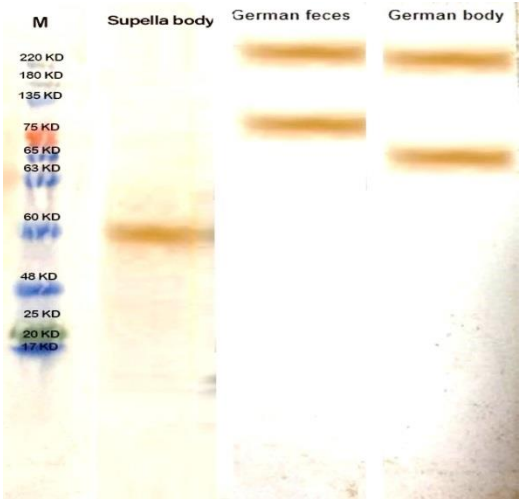

Figure 3: Western blot test, nitrocellulose plates showing protein bands (IgE), from left: supella (brown-banded) cockroach body, German cockroach feces, and finally, German cockroach body

Table 3: Weight of IgE protein bands (antibodies) produced in mouse serum by Western Blot.

Sample	Molecular weight(kDa)
German Cockroach body	65-180
German Cockroach feces	75-220
Supella Cockroach body	60
Supella Cockroach feces	0

In this study using a Western blot test with mouse anti-IgE, samples from the feces and body of German cockroach *(Blattella germanica*) and brown-banded cockroach (*Supella longipalpa*) species were injected into mice as allergens to stimulate the immune system, which primarily produced IgE antibodie except Supella feces. Also, by performing a Western blot test with mouse anti-IgE, it was observed that primarily the allergen of the brown-banded cockroach in the immune system of the mouse produced IgE antibody (weighing 60 kDa), which according to the mentioned sources, indicates that it can have the potential and probability of being allergenic. The IgE antibody is an antibody that is produced in allergens in the immune system. No band was observed for the feces of the brown-banded cockroach. This could be due to a loss of protein in the mouse serum. Serum proteins are rapidly lost for a variety of reasons, including multiple freezing and defrosting. In this test, IgE antibodies weighing 75 and 220 kDa were also produced for the body of the German cockroach, which weighed 75 kDa, indicating an allergen. IgE antibodies weighing 65 and 180 kDa were observed for the feces of this species. Previous studies have shown that cockroach allergens are present in house dust mites, which significantly increase asthma allergens, especially in large urban areas (Bernton and Brown 1967, Twarog et al. 1977, Hulett and Dockhorn 1979, Pollart et al. 1989).

Thangam Sudha et al. (2007) used ELISA measurements to study serum ode IgE antibodies (1.4) in patients with cockroach allergies, measured by injection of American cockroach solutions. In our study, the ELISA results of OD of the American cockroach body were above 2, greater than that value. Furthermore, the OD obtained for Sapla cockroaches was 2.928, and for Sapla feces, 2.55. In the same study, using the SDS-PAGE-Western blot technique, the total weight of American commercial cockroach allergen proteins was measured with 10% gel, and bands with molecular weights of 97, 66, and 88 kDa were observed (Thangam Sudha et al. 2007). In that study with 10% gel, the total weight of proteins measured by SDS-PAGE of the body of the American cockroach and the protein bands weight were 48, 68 and 85 kDa. The results of this study are close to our results. In our study, the total body weight of the German cockroach was 85, 75 kDa and the feces of 100, 85 kDa, respectively; for the Sapella cockroach, it was 75-35-60 and for Supella cockroach feces, this number was 63 kDa. The two species are similar to the results of Thangam's study(Thangam Sudha et al. 2007).

Studies by Earlier et al in 2002 showed 28 German cockroach body protein bands on SDS-PAGE gel. Patterson and Slater (2002) showed that commercial American cockroach allergens are highly variable in the number of bands and protein contents, which can be due to the material preparation method or storage buffer conditions. Differences in the molecular weight of IgE proteins of the cockroach extracts can be due to a variety of allergic compounds as well as sources of serum (Melén et al. 1999). In our study, the body of a German cockroach had about 18 bands in SDS-PAGE and about 8 bands in Western blot. The supella cockroach had 12 protein bands in SDS-PAGE and 1 protein band in Western blot.

In a 2007 study by Morgan et al., The body and feces extracts of the German Cockroaches contained several proteins with bands reported from the serum of a person with allergic symptoms and exposure to these cockroaches. Also, among the 15 susceptible patients at risk being exposed to cockroaches, in terms of skin test, they have been positive, IgE bands were not shown for any of the cockroach feces extracts and had very weak bands, with only 1 35Kd band shown (Melén et al. 1999). This is consistent with our study results of the absence or weakness of the protein band for the Supella cockroach, where the very specific IgE band was not observed for

brown-banded (Supella) cockroach feces. In this study, the measured total protein German cockroach extracts ranged from 0.1 to 24.8 (Morgan et al. 2007). Measurement of total protein concentration by the Bradford test in our study showed a concentration of 1 to 20, which is also consistent with Morgan et al. (2007).

4. Conclusion

According to the results of the ELISA and Western blot tests, it was determined that the cockroach Supella (brown-banded) has allergenic potential. A comparison of the cockroach OD showed that more antibodies, both for feces and body, were produced in the mouse serum than in other cockroaches. A Western blot test also showed that the body of this cockroach produced IgE antibodies in the serum of mice, which can be an important orogenic factor for humans.

Conflict of interest

The authors declare no competing interests.

Acknowledgment

Not Applicable.

Ethical approval

The ethics committee of the Tehran University of Medical Sciences code is 9311263003.

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References

[1] Adler, V. E. (1985). "A highly effective attractant for the brownbanded cockroach (Orthoptera: Blattellidae)." Journal of Environmental Science & Health Part A 20(8): 839-844. https://doi.org/10.1080/10934528509375262

[2] BERNTON, H. and H. Brown (1967). "Cockroach allergy II: the relation of infestation to sensitization." Southern medical journal 60(8): 852-855. https://doi.org/10.1097/00007611-196708000-00012

[3] Carlson, J. C., F. A. Rabito, D. Werthmann and M. Fox (2017). "The Distribution and Movement of American Cockroaches in Urban Niches of New Orleans." Clin Pediatr (Phila) 56(11): 1008-1012. https://doi.org/10.1177/0009922817701169

[4] Cochran, D. G., & World Health Organization. (1999). Cockroaches: their biology, distribution and control (No. WHO/CDS/CPC/WHOPES/99.3). World Health Organization. https://doi.org/10.1016/s0965-1748(99)00073-9

[5] Eggleston, P. A., D. Rosenstreich, H. Lynn, P. Gergen, D. Baker, M. Kattan, K. M. Mortimer, H. Mitchell, D. Ownby and R. Slavin (1998). "Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma." Journal of Allergy and Clinical Immunology 102(4): 563-570. https://doi.org/10.1016/s0091-6749(98)70272-6

[6] Esty, B., P. Permaul, K. DeLoreto, S. N. Baxi and W. Phipatanakul (2019). "Asthma and Allergies in the School Environment." Clin Rev Allergy Immunol 57(3): 415-426. https://doi.org/10.1007/s12016-019-08735-y

[7] Gelber, L. E., L. H. Seltzer, J. K. Bouzoukis, S. M. Pollart, M. D. Chapman and T. Platts-Mills (1993). "Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital." American Review of Respiratory Disease 147: 573-573. https://doi.org/10.1164/ajrccm/147.3.573

[8] Gore, J. C. and C. Schal (2007). "Cockroach allergen biology and mitigation in the indoor environment." Annu Rev Entomol 52: 439-463. https://doi.org/10.1146/annurev.ento.52.110405.091313

[9] Gould, G. E. and H. O. Deay (1940). "The biology of six species of cockroaches which inhabit buildings." The Biology of six Species of Cockroaches which inhabit Buildings.(451). https://doi.org/10.15587/2312-8372.2012.4718

[10] Hashemi-Aghdam, S. S. and M. A. Oshaghi (2015). "A Checklist of Iranian Cockroaches (Blattodea) with Description of Polyphaga sp as a New Species in Iran." J Arthropod Borne Dis 9(2): 161-175. https://doi.org/10.18502/jad.v14i4.5279

[11] Hubner-Campos, R. F., R. N. Leles, J. Rodrigues and C. Luz (2013). "Efficacy of entomopathogenic hypocrealean fungi against Periplaneta americana." Parasitol Int 62(6): 517-521. https://doi.org/10.1016/j.parint.2013.07.013

[12] Hulett, A. and R. Dockhorn (1979). "House dust, mite (D. farinae) and cockroach allergy in a midwestern

population." Annals of allergy 42(3): 160-165. https://doi.org/10.1007/bf02914980

[13] Kambhampati, S. (1995). "A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes." Proc Natl Acad Sci U S A 92(6): 2017-2020. https://doi.org/10.1073/pnas.92.6.2017

[14] Karsonova, A., K. Riabova, S. Villazala-Merino, R. Campana, V. Niederberger, J. Eckl-Dorna, R. Fröschl, T. Perkmann, Y. V. Zhernov and O. G. Elisyutina (2020). "Highly sensitive ELISA-based assay for quantification of allergen-specific IgE antibody levels." Allergy 75(10): 2668. https://doi.org/10.1111/all.14325

[15] Leishangthem, A., D. Venkatesh, P. Kanni and N. P. Komarla (2021). "Cockroach Allergy, Allergen Based Immuno Diagnostic and Specific Immunotherapy: Present Status and Future Trends." RGUHS Journal of Medical Sciences 11(3). https://doi.org/10.26463/rjms.11_3_8

[16] Melén, E., A. Pomés, L. D. Vailes, L. K. Arruda and M. D. Chapman (1999). "Molecular cloning of Per a 1 and definition of the cross-reactive Group 1 cockroach allergens." Journal of allergy and clinical immunology 103(5): 859-864. https://doi.org/10.1016/s0091-6749(99)70430-6

[17] Morgan, M. S., L. G. Arlian, J. A. Bernstein and J. A. Yoder (2007). "Allergenicity of the Madagascar hissing cockroach." Ann Allergy Asthma Immunol 98(3): 258-261.

[18] Nasirian, H. (2010). "An overview of German cockroach, *Blattella germanica*, studies conducted in Iran." Pak J Biol Sci 13(22): 1077-1084. https://doi.org/10.1016/s1081-1206(10)60715-6

[19] Nasirian, H. (2016). "New aspects about Supella longipalpa (Blattaria: Blattellidae)." Asian Pacific Journal of Tropical Biomedicine 6(12): 1065-1075. https://doi.org/10.1016/j.apjtb.2016.08.017

[20] Nasirian, H. and A. Salehzadeh (2019). "Control of Cockroaches (Blattaria) in Sewers: A Practical Approach Systematic Review." J Med Entomol 56(1): 181-191. https://doi.org/10.1093/jme/tjy205

[21] Patterson, M. and J. Slater (2002). "Characterization and comparison of commercially available German and American cockroach allergen extracts 1." Clinical & Experimental Allergy 32(5): 721-727. https://doi.org/10.1046/j.1365-2222.2002.01397.x

[22] Pollart, S. M., M. D. Chapman, G. P. Fiocco, G. Rose and T. A. Platts-Mills (1989). "Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits." Journal of Allergy and Clinical Immunology 83(5): 875-882. https://doi.org/10.1016/0091-6749(89)90100-0

[23] Rodriques, A. (2021). Molecular and Proteomic Characterization of Bla g 2 Allergen in the German Cockroach (Blattella germanica L.), Purdue University Graduate School. https://doi.org/10.1021/acs.jafc.2c05625.s001

[24] Sedghiany, A. A. H.-B. S. (2000). Iranian cockroaches. Tehran University of Medical Sciences and Health Services Publications: 110. https://doi.org/10.18869/acadpub.aums.1.1.13

[25] Thangam Sudha, V., N. Arora, S. Sridhara, S. N. Gaur and B. P. Singh (2007). "Biopotency and identification of allergenic proteins in Periplaneta americana extract for clinical applications." Biologicals 35(2): 131-137. https://doi.org/10.1016/j.biologicals.2006.07.004

[26] Thangam Sudha, V., D. Srivastava, N. Arora, S. N. Gaur and B. P. Singh (2007). "Stability of protease-rich Periplaneta americana allergen extract during storage: formulating preservatives to enhance shelf life." Journal of clinical immunology 27(3): 294-301. https://doi.org/10.1007/s10875-007-9078-y

[27] Twarog, F. J., F. J. Picone, R. S. Strunk, J. So and H. R. Colten (1977). "Immediate hypersensitivity to cockroach: isolation and purification of the major antigens." Journal of Allergy and Clinical Immunology 59(2): 154-160. https://doi.org/10.1016/0091-6749(77)90218-4

[28] Vazirianzadeh, B., R. Dehghani, M. Mehdinejad, M. Sharififard and N. Nasirabadi (2014). "The first report of drug resistant bacteria isolated from the brown-banded cockroach, Supella longipalpa, in ahvaz, south-western Iran." Journal of arthropod-borne diseases 8(1): 53. https://doi.org/10.5812/jjm.10721