Arıcılık Araştırma Dergisi, Cilt:10, Sayı:1, 2018, Sayfa:9-14 ISSN: 2146-2720

ANTIMICROBIAL ACTIVITIES OF NEST MATERIALS DOLICHOVESPULA SAXONICA (FABRICIUS,1793) (HYMENOPTERA: VESPIDAE) IN TURKEY

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ABSTRACT

Organization of bee societies for many years is of interest to many study topic. In this study the antimicrobial activity of diluted with ethanol five fraction nest was assayed in vitro by agar disc diffusion method against 8 bacteria and 2 fungi species.

The ethanol extracts of almost all the nest exhibited antibacterial antifungal activity towards one or another bacterium against to all of microorganism used in this study. The maximum antibacterial antifungal activity was shown by diluted 25 µL (2.5 mg), 20 µL (2 mg), followed by and 15 µL (1.5 mg), respectively. The antimicrobial activities of the extracts of bees nest against bacteria were more effective than those against fungi.

The obtained results show that nest may be used as possible natural, antimicrobial agents to control various human, animal and plant diseases.

Keywords: Dolichovespula saxonica, Wasps nests, Antimicrobial activity

DOLICHOVESPULA SAXONICA (FABRICIUS,1793) (HYMENOPTERA: VESPIDAE) YUVA MATERYALLERiNiN ANTiMiKROBiYAL AKTiViTELERİ

ÖZET

Arı toplumlarının uzun yıllar organizasyonu birçok çalışmaya konu olmuştur. Bu çalışmada yuva materyalinin etanol ile hazırlanan 5 farklı dilüsyonunun antimikrobiyal aktivitesi 8 bakteri ve 2 fungus türüne karşı disk difüzyon metodu ile invitro olarak belirlenmiştir.

Yuva materyallerinin etanol ekstraktlarının bütün konsantrasyonları hemen hemen tüm mikroorganizmalara karşı antibakteriyal ve antifungal etki göstermiştir. Maksimum antibakteriyal ve antifungal etki sırasıyla 25 µL (2,5 mg), 20 uL (2 mg), ardından 15 µL (1,5 mg)'lik dilüsyonlarda görülmüştür. Yuva ekstraktların bakterilere karşı olan antimikrobiyal aktiviteleri, mantarlardan daha fazla etkili olduğu görülmüştür.

Elde edilen sonuçlar, yuva materyalinin insan, çeşitli hayvan ve bitki hastalıklarını kontrol etmek için mümkün olan doğal, antimikrobik maddeler olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Dolichovespula saxonica, Arı yuvası, Antimikrobiyal aktivite

1. Introduction

Social wasp nests serve as a place for rearing brood and the centre for their nesting activities (Starr, 1991; Jeanne, 1997). Wasps collect woody fibers, minute vegetable chips, plant which hairs and mud, they masticate and mix with oral secretion to construct the nest with a variety of architectural design (Jeanne, 1997). This salivary secretion is also used to physically maintain their nests. Wasps frequently smear the surface of their nests with the secretion to protect the nest from rain and weathering. It has been reported recently in two Japanese Dolichovespula wasps, Dolichovespula saxonica (D. saxonica) that a large amount of the secretion is used for construction and maintenance of pre-emergence nests (Yamane et al. 1998). Since the oral secretion is composed primarily of protein in most wasp genera so far analyzed (Jeanne, 1997). It is presumed that the cost for secretion production is very high (Maschwitz et al. 1990). The amino acid composition of protein in the nests has been examined in D. saxonica (Mcgovern et al. 1998). These results showed that alanine, glycine and serine were major components in the nests of these species. In addition, they also suggested that the evolution of the chemical nature of the oral secretion, such as amino acid composition was also important for social evolution in the family Vespidae. There is little published data on the effects of bee nests content on Vespadea. Only a few studies on the antimicrobiyal activity of bee nests content of D. saxonica its own species demonstrated significant results In addition, the degree of antimicrobial activity of nests on individuals of their own species is unknown. Many social insects have developed defensive systems that prevent infections within their colonies. For example, bee propolis and royal jelly present antimicrobial properties and the fecal pellets of termites inhibit the development of fungal pathogens (Anderson et al. 2011; Rosengaus et al. 1998). Concerning ants, most species possess met pleural glands on the thorax whose secretions, spread over individuals and throughout the nest, have a broad spectrum of antimicrobial action. This activity might be involved in the processing of antibacterial peptides, or other peptides, which may play a role in conditioning the host to ensure the survival and development of parasitoid progeny through the limited hydrolysis of peptide precursors (Blum et al. 1985). Venoms of honey bees, wasps and hornets, including D. saxonica possess antimicrobial peptides; however, their natural functions must be further clarified (Anderson et al. 2011; Monterio et al. 2009; Banks et al. 1986). In the present study for that purpose, the total phenolic contents, antioxidant potentials, Biochemical analyses, antimicrobial activities and some physical features and elemental composition of the nests of bee nest samples obtained from East Black Sea Region was investigated.

2. Materials and Methods

2.1. Nest collectionand extraction

The nest of D. saxonica collected in Trabzon (39°43’ E, 41°00’ N,Turkey) during July –August 2015 Region of East Black Sea (Yildirim et al. 1992) Larvae, pupae and eggs were removed from the nest. Small fragments were cut from the comb for observation. The nests were stored in the Entomology Laboratory at Biology Department of Ordu University, Turkey.

The nest samples were extracted with 95 % ethanol at room temperature. The extracts were kept at 4 °C for a day, and they were filtered through a 0.45 µm membrane filter. Obtained filtrates were stored at -20 °C until analysis (Ertürk et al. 2009).

2.2. Microorganisms tested and culture media

Strains of bacteria and fungus were obtained from ATCC (American Type Culture Collection). The antimicrobial activity of honey samples was studied using

ten microorganisms. Three gram-positive: Staphylococcus aureus (S. aureus) ATCC®25923, Micrococcus luteus (M. luteus) B1018, Basillus subtillis (B. subtillis) B209, and five gram negative: Proteus vulgaris (P. vulgaris) B123, Klebsiella pneumonia (K. pneumoniae) ATCC®13883, Pseudomonas aeruginosa (P. aeruginosa) ATCC®27853, Streptomyces murinus (S. murinus) ISP 5091, Yersina enterocolitica (Y. enterocolitica) ATCC®27729, and two fungus Candida albicans (C. albicans) ATCC®10231, Aspergillus niger (A. niger) ATCC 9642, species. Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck) and Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and fungal cells, respectively. The concentrations of bacterial suspensions were adjusted to 108 cells/mL, and fungal suspension to 107cells/mL.

2.3. Antibacterial and Antifungal assay

For determination of antibacterial and antifungal activity, diffusion disk plates method was used [13]. For this purpose, first of all, bacterial strains grown in MHB medium for 24 h, at 37°C and fungal strains were also grown in SDB medium for 27 h, at 48 °C. Overnight cultures were diluted with broth and the final bacterial and fungal cell concentrations were adjusted to 108 and 107 cells/mL by measuring spectrophotometrically at A600 nm, respectively. 20 mL of MHA and SDA medium was poured into each 15 cm Petri dish and allowed to solidify. Each of the extract sample of nest was tested in concentrations of 1, 0.5, 0.25, and 0.125 g/ml. From the test solutions 30 µL of each diluted suspension was placed over agar in petri dishes and dispersed. Then sterile paper discs (Oxoid, CT09988, 6 mm diameter) were placed on agar to load 30 µL of each nest samples. Inhibition diameters were determined after incubation for 24 h at 37 °C and 27 h at 48 °C for antibacterial and antifungal activities, respectively. All tests were made in triplicate.

2.4. Statistical analysis

Values shown in tables were means ± standard deviations of three parallel measurements. The values showed the simple harmony. They were demonstrated by visual (Table 1). The SC50 values were calculated from linear regression analysis (MsExcel 2003). Data were tested using SPSS (SPSS Inc., Chicago, Illinois, USA). Statistical analysis of the results was based on Mann–Whitney U-test and Pearson correlation analyses. Differences of $p<0.05$ were considered to be significant.

3. Result

The antibacterial activity of diluted with alcohol five fraction nest was assayed in vitro by agar disc diffusion method against 8 bacterial and 2 fungi species. Table 1 summarizes the microbial growth inhibition of ethanol extracts of the screened beeswax nest. The five dilutions nest ethanol extract showed Antibacterial antifungal activity. On the other hand, ethanol extracts of almost all the nest exhibited antibacterial antifungal activity towards one or another bacterium against to all of microorganism used in this study. The maximum antibacterial antifungal activity was shown by diluted 25 µL (2.5 mg) of nest extracts 20 µL (2 mg), followed by and 15 µL (1.5mg), respectively. The ethanol extracts of the investigated diluted 25 µL (2.5 mg) of nest showed maximum antibacterial and antifungal activity against Gram-negative Proteus vulgaris, Gram-positive Bacillus subtillis and Candida albicans. The ethanol extracts of the investigated five diluted of nest showed minimum antibacterial and antifungal activity against Gram-negative

Pseudomonas aeruginosa, Gram-positive Staphylococcus aureus and Aspergillus niger. The antimicrobial activities of the extracts of bees nest against bacteria were more effective than those against fungi, which is similar to the results of by (Ertürk et al. 2003).The significant antibacterial activity of the active diluted nest extracts was comparable to the standard antimicrobics, ampicillin (30 µg/disc), cefazolin (30 µg/disc) and Nystain (30 µg/disc),

Tablo 1. Results of antimicrobial screening of Dolichovespula saxonica's nest extracts determined by the agar diffusion method (inhibition zone in mm)

Nest extracts mg/mL	Y.e.	A. n.	S. m.	K. p.	S. a	M. l.	C. a.	B. s.	P. v.	P. a.
5 µL (0.5 mg)	6.00±0.00	6.00±0.00	12. 33±0.57	6.00±0.00	6.00±0.00	13.00±0.00	10.33±0.57	6.00±0.00	10.33±0.57	6.00±0.00
10 µL (1mg)	6.00±0.00	6.00±0.00	14.33±0.57	10.33±0.57	6.00±0.00	14.33±0.57	12.33±0.57	6.00±0.00	12.33±0.57	6.00±0.00
15 µL (1.5mg)	6.00±0.00	6.00±0.00	22.33±0.57	21,00±0.00	6.00±0.00	14.33±0.57	12.33±0.57	6.00±0.00	13.00±0.00	6.00±0.00
20 µL (2 mg)	6.00±0.00	12. 33±0.57	25.33±0.57	22.00±0.00	10.33±0.57	21,00±0.00	14.33±0.57	6.00±0.00	1433±0.57	6.00±0.00
25 µL (2.5 mg)	6.00±0.00	12. 33±0.57	25.33±0.57	34.33±0.57	10.33±0.57	27.00±0.00	27.33±0.57	6.00±0.00	1633±0.57	6.00±0.00
Alkol	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00,	6.00±0.00	6.00±0.00
Ampicillin	15.33±0.57	NT	11.33±0.57	13.33±0.57	15.00±0.00	6.00±0.00	NT	36.33±0.57	28.33±0.57	27.33±0.57
Cefazolin	15.33±0.57	NT	6.00±0.00	11.00±0.00	1633±0.57	35.33±0.57	NT	38.33±0.57	6.00±0.00	24.33±0.57
Nystain	NT	14. 00±0.00	NT	NT	NT	NT	15.33±0.57	NT	NT	NT

Microorganisms: Y.e.: Yersinia enterocolitica, A.n.: Aspergillus niger, S.m: Streptomyces murinus, K.p.: Klebsiella pneumoniae, S.a.: Staphylococcus aureus, P.a.: Pseudomonas aeruginosa, M.l.: Micrococcus luteus, C.a.: Candida albicans, B.s.: Bacillus subtilis, P.v.: Proteus vulgaris, NT: Not test

4. Discussion

Social insects face especially high risks from parasites and pathogens, due to crowded living conditions and the potential that closely related nest mates will share same vulnerabilities against specific pathogens (Scheremmer et al. (1985). Several species across the animal kingdom may use these plant-produced resins to reduce effects of parasites and pathogens. One well-described example, Formica paralugubris, a Swiss wood ant, mixes resin globules from coniferous trees with nest material, and this resin decreases the number of total microorganisms in the nest (Christe et al. 2003). The chemistry and choice of nest resins useful as repellants of natural enemies has notbeen studied, although the antibacterial propertiesof resins are well known (Lokvam et al. 1999; Langenheim et al 2003).

The chemical consistency of bees nest is highly dependent on the flora of the region from which it is collected. In contrast to nest of continental Europe, different bees nest has a different botanical origin due to unique flora of country that has developed as a result of its geographical position. The country flora presents a generally known biodiversity with a high percentage of endemic plants (Spradbery, 1973).

In conclusion, beeswax nest extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial and antifungal compounds.

The nest itself is composed of a paper-pulp mixture created by female workers chewing up dead bark, trees, or plant matter that is closely surrounding them and mixing it in with their saliva. To build the actual comb, saliva is used as a cement to piece together organic and inorganic materials that are readily available to the colony. This cement not only holds together the comb, but also protects the comb from being damaged by water. In these same nests studied in Turkey, fiber content was 23 percent while 77 percent was hornet saliva. Just for these nests, the resulting water absorption capacity turned out to be optimal: 100 percent (Silva et al. 2006).

5. References

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