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Orijinal araştırma (Original article)

Natural cellular immunity in field-collected insects from Hatay province by assessing nodulation[1]

Hatay yöresinden toplanan böceklerde oluşan hücresel bağışıklığın nodülasyon testi ile tespiti

Hasan TUNAZ[2*] **Ali A. IŞIKBER[2]** **M. Kubiay ER[2]**

Summary

Natural microbial infections to insects collected from agrarian fields surrounding Hatay Province,Turkey were determined by assessing nodulaiton which is one of insect cellular immunity. After identifying insect specimens, the insects were dissected to assess numbers of nodules. Nodulation is one of the predominant cellular immune reactions to microbial infections and the nodules are permanently attached to internal surfaces of the insects. We collected about 660 insect specimens for nodulation and found nodules in 99 % out of all the examined specimens. Appearance of examined insects was healthy. Number of nodules in each insect ranged from 1 to 118. Our results indicated that insects are regularly challenged by microbial infections in nature and insect immune systems can limit the host range and effectiveness of microbial agents deployed in biological control programs. Therefore, undertsanding insect immune systems is important for the efficacy and use of microbial pesticides in biological control of insects.

Keywords: Insect immunology, naturally occurring infections, nodulation

Özet

Bu çalışma Hatay yöresinden toplanan böceklerde doğada mikrobiyal hastalıklara karşı oluşan hücresel bağışıklığı orataya koymak için yürütülmüştür. Böcek türleri teşhis edildikten sonra doğal mikrobiyal enfeksiyonlara karşı oluşan hücresel bağışıklıklardan nodülasyon testi için böcekler buz üzerinde bayıltılarak mikroskop altında vücutları kesilerek (dissect) açılmıştır. Böceklerde nodülasyon mikrobiyal enfeksiyonlara karşı oluşturalan hücresel bağışıklardan birisi olup böceğin iç organlarında görülmektedir. Çalışmada yaklaşık 660 böcek bireyi nodülasyon reaksiyonu için test edilmiş ve test edilen böceklerde % 99 oranında nodüle rastlanmıştır. Böcek bireylerinde nodül sayısı 1 ile 118 arasında değişmiştir. Bu sonuçlar doğada böceklerin mikrobiyal enfeksiyonlarla karşı karşıya olduğunu, böceklerin bu enfeksiyonların üstesinden gelebildiğini ve doğada böcek bağışıklığını anlamanın zararlı böceklerle mücadelede kullanılacak mikrobiyal pestisitlerin etkinliğinin ve kullanımının artırılması bakımından önemli olduğunu ortaya çıkarmıştır.

Anahtar sözcükler: Böcek bağışıklığı, doğal oluşan enfeksiyon, nodülasyon

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[2] KSU, Faculty of Agriculture, Plant Protection Department, Kahramanmaraş, Turkey
* Corresponding author (Sorumlu yazar) email: htunaz@ksu.edu.tr
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Introduction

Insects are regularly infected by entomopathogens including viruses, fungi and bacteria in nature. These entomopathogenic microbes can regulate insect populations in nature (Lacey et al., 2001). Insect diseases and the possibilities of using insect disease agents in biological control of insects have been known since 1957 (Steinhaus, 1957; Tanada, 1959). Currently entomopathogens including viruses, fungi, bacteria, protozoans, parasitoids, nematodes and predators are commercially used in biocontrol of insect pests, weeds and plant diseases.

Lomer (1999) mentioned that the success and failure of biocontrol of insects mostly depend on various factors, including costs, the context of comprehensive IPM programs, education of users, government activities, as well as political and environmental concerns. However, biological issues are important for successful biocontrol of insects. These biological issues include the ecological level of microbe-host population dynamics and the molecular and cell biology of host defense mechanisms, such as, insect immunity, one of crucial barriers to the success of insect biocontrol programs.

Insect immunity is comprised of a number of systems. These are physical barriers, cellular and humoral immunity. Integument and alimentary canal of insects protect them from microbial invasions as physical barriers. Once microbes pass the physical barriers surrounding insect bodies, insect cellular immunity, including phagocytosis and nodule formation, takes action against microbial invaders (Lavine & Strand, 2002; Stanley & Miller, 2006). Finally, the humoral immune system of insect, which involves anti-microbial peptides, takes some hours to activate (Lemaitre & Hoffmann, 2007). Insects also express behavioral fevers following infection. The combined arsenal of immune effector mechanisms allows insects to either stifle infections at their onset or to overcome invasions and infections.

We cannot figure out which insect immunity functions protect insects from microbial infections in nature and we do not know how insect immunity can influence biocontrol programs. However, Ouedraogo et al. (2004) mentioned that insect febrile reactions alone may affect the effectiveness of fungal biocontrol agents in laboratory and field experiments. Therefore, insect defense mechanisms can limit the effectiveness of microbes deployed for biocontrol of insect. Now, we have also known that most insects in agrarian habitats of Kahramanmaraş and Adana/Turkey experience naturally occurring infections and the insects recover from invading microbes with fast-acting cellular defense actions, including nodule formation (Tunaz & Stanley, 2009; Tunaz et al., 2015). Their work also showed that insect cellular defense mechanism, nodulation, was affected by various factors including location, season, altitude, taxonomic position, and biological stage of insects collected. Hence, to broaden this area, in this paper we investigated natural microbial infections to field-collected insects from Hatay Province, another different geographic area, by assessing nodulation, which is one of the insect cellular immune reactions. For this purpose, we hypothesized that most insects in nature experienced microbial infections and they overcome the infections. If the hyporhesis is true, insect immunity can limit the effectiveness of microbial-based biocontrol programs. Here is the report of our research results to test the hypothesis.

Materials and Methods

Organisms

Insects were collected from Hatay Province of Turkey from April, 2011 until September, 2013. Insects were collected either by hand or a net. We identified and recorded the collected insect species and their biological stages, the collection sites and site altitudes. We transferred the insects to the laboratory (20 ± 1 ^{o}C, 60 ± 5 % RH) at Kahramanmaras Sütçü İmam University. The insects were further identified to mostly species level. Voucher insect specimens were kept in the Entomology Collection, Kahramanmaras Sütçü İmam University.

Assessing nodulation

For nodulation assay, identified live insects were anesthetized by chilling on ice and then their hemocoels were exposed. We counted nodules under a stereo microscope at 45x. The determination of nodules and level of cellular immune response are based on Miller & Stanley (1998), who identified nodules as distinct, melanized, brownish-black color nodules and the number of nodules reflected the extent of cellular immune response to infections. The internal tissues including gonads, fat bodies and others were carefully probed for previously unseen nodules.

Statistical analysis

Data on nodulation were analyzed using the General Linear Models procedure, and mean comparisons were made using Duncan test (SAS Institute Inc., 1989).

Results

A total of 66 different insect species collected during winter, spring, summer and fall of 2011, 2012 and 2013 were checked for nodulation (Tables 1-3). Thirteen different insect species belonging to Lepidoptera, Hemiptera, Coleoptera, Orthoptera and Phasmida in 2011, fourthy-four different insect species belonging to Lepidoptera, Hemiptera, Coleoptera, Orthoptera, Diptera, Hymenoptera, Odonata and Phasmida in 2012, fifty-six different insect species belonging to Lepidoptera, Hemiptera, Coleoptera, Orthoptera, Diptera, Hymenoptera, Odonata, Dermaptera and Phasmida in 2013 were collected from various plants and soil and were checked for nodulation (Tables 1-3). We saw nodules in 99 % of the 660 specimens examined, although the range of nodules/specimen (from 1 nodule/insect to >118 nodules/insect) was quite wide.

Generally, more nodules were seen in the insects associated with soil than in those collected from plants (Table 4). Specifically, we observed higher number of nodules in orthopteran specimens and sunn pest adults. There were more nodules in orthopteran specimens and sunn pest adults associated with soil (Tables 1- 3). It was also noted that the overwintered generations of *Ostrinia nubilalis, Sesamia nonagrioides* and *Eurygaster integriceps* had much more nodules compared to new genaration larvae and adults (Table 1- 3). Examining insect orders for nodulation assay, significantly more nodules were recorded from orthopteran species than lepidopteran, hemipteran and coleopteran species (Table 6), which is logical because orthopteran species were mostly collected from soil. There were no nodulation differences in number among biological stages of insects. It was recorded statistically similar numbers of nodules in larvae, nymphs and adults of insect species (Table 5). While the insects collected at lower altidutes had more nodules than the insects collected at higher altitudes in 2011, there were no nodulation number differences in collecting altitudes of insects in 2012 and 2013 (Table 7). Putting together, insect orders in contact with soil are probably the main associations with higher number of nodules. However, the actual occurrence of natural infection may be a random event with no predominant patterns. On the other hand, the data indicate virtually all insects had experienced infection(s) in nature.

Table 1. Average numbers of nodules in insects collected from various sources in Hatay Province in 2011. Values indicate numbers of discrete nodules ± SEM. Collection dates are dd/m/yr

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Lepidoptera				
Pieris brassicae	59.4 ± 18.6	Weeds	Larvae	28/04/11, 350m
Pieris brassicae	12.3 ± 3.6	Cabbage	Larvae	21/10/11, 420m
Pieris rapae	2.7 ± 0.92	Cabbage	Larvae	21/10/11, 420m
Ostrinia nubilalis	3.6 ± 0.94	Corn stalk	Larvae	21/10/11, 420m
Sesamia nonagrioides	6.1 ± 1.84	Corn stalk	Larvae	21/10/11, 420m
Heliothis armigera	12.2 ± 5.22	Cabbage	Larvae	21/10/11,420m
Hemiptera				
Eurygaster integriceps	11.8 ± 10.4	Wheat	New generation adults	01/06/11,150m
Eurygaster integriceps	117.5 ± 38.2	Wheat	Wintered adults	28/04/11,150m
Nezara viridula	5.7±1.19	Beans	Adults	21/10/11, 420m
Eurydema ornatum	2.7±0.49	Radish	Nymphs	21/10/11, 420m
Eurydema ornatum	3.4±0.78	Radish	Adults	21/10/11, 420m
Coleoptera				
Capnodis spp.	11.1 ± 3.4	Apricot	Adults	23/04/11, 500m
Coccinella semptempunctata	2.5±0.88	Weeds	Adults	21/10/11, 420m
Orthoptera				
Acrididae	6 ± 2.57	Weeds	Adults	21/10/11 420 m
Gryllus assimilis	20.8 ± 5.14	Soil	Adults	21/10/11, 420m
Phasmida				
Gratidia sp.	28.5 ± 7.90	Soil	Adults	21/10/11 420 m

Table 2. Average numbers of nodules in insects collected from various sources in Hatay Province in 2012. Values indicate numbers of discrete nodules ± SEM. Collection dates are dd/m/yr

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Lepidoptera				
Arctia sp.	13.9 ± 1.9	Weeds	Larvae	26/03/12, 420m
Pieris brassicae	48.6 ± 17.3	Weeds	Larvae	23/04/12, 300m
Pieris brassicae	9.7 ± 1.64	Cabbage	Larvae	19/11/12, 411m
Pieris brassicae	20.5 ± 2.4	Cabbage	Larvae	24/12/12, 410m
Papilio machaon	20.7 ± 2.6	Weeds	Larvae	20/06/12,,270m
Thaumetopoea pityocampa	14.2± 4.3	Pine	Larvae	20/6/12,700m
Colias croceus	1.44 ± 0.41	Weeds	Adults	12/07/12,,310m
Ostrinia nubilalis	5.61.6	Corn stalk	Larvae	19/11/12, 334m
Sesamia nonagrioides	8.6 ± 3.52	Corn stalk	Larvae	19/11/12, 334m
Pieris rapae	1.2 ± 0.37	Cabbage	Adults	22/10/12, 380m
Pieris rapae	11.7 ± 2.1	Cabbage	Larvae	19/11/12, 411m
Pieris rapae	22.5 ± 4.5	Cabbage	Larvae	24/12/12, 410m
Pieris rapae	5.5 ± 1.5	Cabbage	Larvae	21/01/13, 410m
Pieris rapae	6.7 ±0.73	Cabbage	Adults	18/02/13, 387m
Helicoverpa armigera	5.42 ± 1.13	Cabbage	Larvae	22/10/12, 350m
Helicoverpa armigera	36.33 ± 5.6	Alfalfa	Larvae	19/11/12,164m
Helicoverpa armigera	25 ± 1.7	Alfalfa	Larvae	24/12/12, 164m
Acronicta spp.	17 ± 1.15	Alfalfa	Larvae	19/11/12, 164m
Coleoptera				
Cantharis sp.	5 ± 1.14	Wheat	Adults	26/3/12, 100m
Cantharis sp.	3.4 ± 0.75	Wheat	Adults	23/04/12,100m
Cantharis sp.	3.1±0.72	Wheat	Adults	18/02/13, 360m
Carabidae	7.5 ± 1.25	Weeds	Adults	28/05/12,420m
Carabidae	17.25 ± 2.91	Weeds	Adults	05/06/12,320m
Coccinella semptempunctata	3.2±0.6	Weeds	Adults	15/05/12, 420m
Coccinella semptempunctata	4.66±1.62	Weeds	Adults	13/06/12, 400m
Coccinella semptempunctata	2.7±0.78	Weeds	Adults	22/10/12, 389m
Coccinella semptempunctata	0.66±0.33	Weeds,	Larvae	19/11/12, 164m
Coccinella semptempunctata	2.6±0.68	Weeds	Adults	19/11/12, 164m
Coccinella semptempunctata	2.5±1.7	Weeds	Adults	24/12/12, 180m
Coccinella semptempunctata	0.9±0.31	Wheat	Adults	18/02/13, 361m
Larinus latus	9.7±1.37	Weeds	Adults	20/06/12, 400m
Oxythyrea cinctella	11.5±0.5	Weeds	Adults	13/06/12, 400m
Hippodemia variegata	0.428±0.2	Weeds	Adults	29/06/12, 270m
Coccinella bipunctata	0.57±0.29	Weeds	Adults	12/07/12, 310m
Hypera variabilis	0.5±0.5	Alfalfa	Adults	24/12/12, 164m
Staphylinidae	17.13±6.24	Soil	Adults	21/01/12, 138m
Hemiptera				
Dolycoris baccarum	5.87 ± 0.87	Weeds	Adults	08/06/12,400m
Dolycoris baccarum	1.6 ± 0.4	Weeds	Nymphs	28/05/12, 420m
Dolycoris baccarum	22.4 ± 6.24	Weeds	Adults	15/05/12,420m
Carpocoris mediterraneus	18 ± 5.56	Weeds	Adults	15/05/12, 420m
Carpocoris mediterraneus	30 ± 6	Weeds	Adults	08/06/12, 40 m
Eurydema ornatum	4.7 ± 1.35	Corn	Adults	26/03/12, 420m
Eurydema ornatum	4.37 ± 0.82	Weeds	Nymphs	8/06/12, 400m
Eurydema ornatum	3.5 ± 0.80	Weeds	Nymphs	12/07/12, 400 m
Eurydema ornatum	3.5±0.5	Alfalfa	Adults	19/11/12, 164m
Cercopidae	0.7 ± 0.26	Weeds	Adults	26/03/12, 100 m
Eurygaster integriceps	105.1 ± 14.078	Soil	Wintered adults	23/04/12, 90m
Eurygaster integriceps	12 ± 1.53	Wheat	New generation adults	20/06/12, 390m
Miridae	5.1 ± 1.7	Weeds	Adults	15/05/12, 320m
Miridae	6.6 ± 4.17	Weeds	Adults	13/06/12, 390m
Aelia rostrata	7.5 ± 2.5	Wheat	New generation adults	20/06/12,390m
Ancyrosoma leucogrammes	10.66 ± 3.71	Weeds	Adults	08/06/12, 381m
Ancyrosoma leucogrammes	2 ± 0.57	Weeds	Adults	12/07/12, 400m
Cicadidae	6.6 ± 1.77	Weeds	Adults	13/06/12, 400 m
Nezara viridula	5.5±0.94	Weeds	Adults	20/6/12, 230m
Rhynocoris sp	10 ± 1.73	Weeds	Adults	13/06/12, 39 m

Table 2. (Continued)

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Orthoptera				
Acrididae	18 ± 3.57	Weeds	Adults	28/05/12 420m
Acrididae	37 ± 1.6	Weeds	Adults	13/06/12 400m
Acrididae	30.5± 5.139	Weeds	Adults	12/07/12 428m
Acrididae	23.87 ± 2.71	Weeds	Adults	22/10/12, 389m
Acrididae	52.3 ± 3.6	Soil	Adults	19/11/12, 175m
Acrididae	54 ± 9.6	Soil	Adults	24/12/12, 385m
Acrididae	39 ± 6.8	Weeds	Adults	21/01/12, 138m
Poecilimon spp.(Tettigoniidae)	38 ± 11.48	Weeds	Nymphs	13/06/12, 400m
Poecilimon spp.(Tettigoniidae)	29.75 ± 6.58	Weeds	Nymphs	29/6/12, 428m
Gryllus bimaculatus	15.2 ± 11	Weeds	Adults	21/01/12, 140m
Gryllus bimaculatus	28.32 ± 3.8	Soil	Adults	18/02/12, 351m
Diptera				
Eristalis tenax	12.5 ± 2.65	Weeds	Adults	04/04/12, 420m
Hymenoptera				
Cephus pygmaeus	4.3 ± 0.66	Weeds	Larvae	28/5/12 420m
Apidae	20.7 ± 2.6	Weeds	Adults	13/06/12,270m
Apis mellifera	5.1 ± 0.79	Weeds	Adults	29/06/12 383m
Bombus sp.	3 ± 0.36	Weeds	Adults	12/7/12 420m
Xylocopidae	13.3 ± 4.5	Weeds	Adults	18/02/13, 380m
Odonata				
Libellulidae	28.5 ± 1.5	Weeds	Adults	29/06/12 400m
Libellula depressa	27.5 ± 3.5	Weeds	Adults	22/10/12, 389m
Libellula depressa	67.26 ± 13.14	Weeds	Adults	19/11/12, 164m
Anax imperator	55.12 ± 16.14	Weeds	Adults	19/11/12, 164m
Phasmida				
Gratidia sp.	9.7 ± 2.78	Weeds	Adults	12/7/12 270m

Table 3. Average numbers of nodules in insects collected from various sources in Hatay Province in 2013. Values indicate numbers of discrete nodules ± SEM. Collection dates are dd/m/yr

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Lepidoptera				
Ostrinia nubilalis	14.8±2.1	Corn stalk	Wintered larvae	21/01/13, 338m
Ostrinia nubilalis	28.6±1.91	Corn stalk	Wintered larvae	18/02/13, 105m
Sesamia nonagrioides	17 ± 3.2	Corn stalk	Wintered larvae	21/01/13, 105m
Sesamia nonagrioides	36 ± 2	Corn stalk	Wintered larvae	18/02/13, 338m
Sesemia nanogrioides	23.2±2.33	Corn	Adults	24/05/13, 90m
Papilio machaon	23±3.1	Weeds	Larvae	21/06/13, 350m
Thaumetopoea pityocampa	41.2±9.3.	Pine	Larvae	28/06/13, 600m
Arctia sp.	14.2±2.1	Weeds	Larvae	24/03/13, 420m
Vanessa cardui	7.2±1.3	Weeds	Adults	12/04/13, 95m
Pieris brassicae	26.1 ± 1.9	Cabbage	Larvae	21/01/13, 410m
Pieris brassicae	38.4 ± 12.3	Weeds	Larvae	19/04/13, 400m
Pieris brassicae	40.2 ± 7.3	Weeds	Larvae	21/06/13, 450m
Pieris brassicae	12.3 ± 4.12	Weeds	Adults	11/07/13, 95m
Pieris brassicae	1.75 ± 0.47	Alfalfa	Adults	23/08/13, 65m
Pieris rapae	5.3 ± 1.2	Cabbage	Adults	5/04/13, 90m
Pieris rapae	8.2 ± 2.1	Cabbage	Adults	12/04/13, 85m
Pieris rapae	11.1 ± 2.03	Alfalfa	Adults	19/07/13, 67m
Pieris rapae	11.2 ± 5.2	Cabbage	Larvae	26/07/13, 470m
Geometridae	10.2±2.3	Alfalfa	Larvae	5/07/13, 100m
Colias crocea	2.1±0.41	Weeds	Adults	11/07/13, 300m
Colias crocea	17.3±4.05	Alfalfa	Adults	19/07/13, 70m
Colias crocea	16.3±3.9	Alfalfa	Adults	2/08/13, 370m
Colias crocea	1.8±0.98	Alfalfa	Adults	16/08/13, 60m
Colias crocea	2.6±0.76	Alfalfa	Adults	23/08/13, 65m
Pontia sp.	9.33±2.02	Alfalfa	Adults	19/07/13, 60m
Aspitates sp.	20.5±7.2	Weeds	Adults	17/05/13, 100m
Helicoverpa armigera	38.2 ± 5.4	Alfalfa	Larvae	8/06/13, 100m
Helicoverpa armigera	8.2 ± 1.17	Alfalfa	Adults	16/08/13, 60m
Helicoverpa armigera	20.16 ± 3.88	Alfalfa	Larvae	23/08/13, 65m
Polyommatus sp.	6 ± 2.3	Alfalfa	Adults	19/07/13, 70m

Table 3. (Continued)

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Coleoptera				
Oxythyrea cinctella	11.14±1.29	Weeds	Adults	3/05/13, 73m
Oxythyrea cinctella	13.1±.4.7	Weeds	Adults	14/06/13, 350m
Coccinella semptempunctata	0.7±0.4	Weeds	Adults	24/03/13 100m
Coccinella semptempunctata	1.9±1.28	Weeds	Adults	26/04/13, 85m
Coccinella semptempunctata	0.6±0.22	Weeds	Adults	3/05/13, 80m
Coccinella semptempunctata	0.37±0.18	Weeds	Adults	10/05/13, 68m
Coccinella semptempunctata	1±0.5	Weeds	Adults	24/05/13, 90m
Coccinella semptempunctata	2.1±0.34	Weeds	Adults	8/06/13, 100m
Coccinella semptempunctata	1.2±0.62	Alfalfa	Adults	26/07/13, 470m
Coccinella semptempunctata	1.2±0.86	Alfalfa	Adults	16/08/13, 420m
Coccinella semptempunctata	1.5±1.5	Alfalfa	Adults	30/08/13, 110m
Larinus latus	9.2±2.8	Weeds	Adults	5/04/13, 100m
Larinus latus	28.66±2.02	Weeds	Adults	10/05/13, 68m
Larinus latus	22.5±2.5	Weeds	Adults	10/05/13, 68m
Larinus latus	10.2±1.9	Weeds	Adults	28/06/13, 410m
Larinus onopordi	31±2.32	Weeds	Adults	3/05/13, 73m
Lixus sp.	17.3±6.4	Weeds	Adults	26/04/13, 85m
Lixus sp.	8.5±5.5	Weeds	Adults	19/07/13, 65m
Cantharis spp.	5.8±2.2	Wheat	Adults	24/03/13 100m
Cantharis spp.	6.4±2.1	Weeds	Adults	12/04/13, 85m
Cantharis spp.	4.8±0.92	Wheat	Adults	19/04/13, 100m
Cantharis spp.	9±1.28	Weeds	Adults	26/04/13, 85 m
Cantharis spp.	3.85±0.76	Weeds	Adults	3/05/13, 73m
Cantharis spp.	22±7	Weeds	Adults	24/05/13, 90m
Phyllopertha horticola	4.2±0.77	Weeds	Adults	26/04/13, 85m
Phyllopertha horticola	20.5±2.06	Weeds	Adults	3/05/13, 73m
Phyllopertha horticola	8.8±0.86	Weeds	Adults	10/05/13, 68m
Gonioctena fornicata	5.3±1.9	Alfalfa	Adults	21/06/13, 450m
Hypera variabilis	3.2±1.2	Alfalfa	Adults	24/05/13, 90m
Hippodemia variegata	12.2±6.7	Weeds	Adults	28/06/13, 600m
Hippodemia variegata	0.62±0.23	Weeds	Adults	5/07/13, 410m
Adalia decempuncatata	0.9±0.4	Weeds	Adults	5/07/13, 410m
Coccinella decemlinata	0.28±0.18	Weeds	Adults	24/05/13, 90m
Coccinella decemlinata	0.6±0.4	Alfalfa	Adults	30/08/13, 110m
Adelia bipunctata	0.71±0.28	Weeds	Adults	24/05/13, 90m
Adelia bipunctata	1.62±0.63	Alfalfa	Adults	11/07/13, 95m
Adelia bipunctata	0.76±0.26	Alfalfa	Adults	16/08/13, 420m
Coccinella undecipunctata	0.4±0.16	Alfalfa	Adults	19/07/13, 67m
Coccinella undecipunctata	0.6±0.21	Alfalfa	Adults	2/08/13, 370m
Hemiptera				
Membracidae	1.1 ± 0.7	Weeds	Adults	28/06/13, 600m
Ancyrosoma leucogrammes	9.9± 3	Weeds	Adults	14/06/13, 350m
Ancyrosoma leucogrammes	8± 2.3	Weeds	Adults	19/07/13, 65m
Ancyrosoma leucogrammes	9± 3.4	Weeds	Adults	2/08/13, 400m
Eurygaster integriceps	91.7 ± 4.65	Wheat	Wintered adults	24/03/13, 75m
Eurygaster integriceps	84.3 ± 9.4	Wheat	Wintered adults	12/04/13, 90m
Eurygaster integriceps	101.2 ± 12.8	Wheat	Wintered adults	19/04/13, 400m
Eurygaster integriceps	89.2 ± 5.13	Wheat	Wintered adults	26/04/13, 85m
Eurygaster integriceps	95.7 ± 5.08	Wheat	Wintered adults	3/05/13, 73m
Eurygaster integriceps	8.8 ± 7.3	Wheat	New generation adults	31/05/13, 73m
Nezara viridula	9±2	Weeds	Adults	8/06/13, 400m
Nezara viridula	12.1±3.8	Alfalfa	Adults	11/07/13, 95m
Nezara viridula	3.2±1.2	Alfalfa	Nymphs	26/07/13, 480m
Nezara viridula	9.1±2.3	Alfalfa	Adults	2/08/13, 60m
Nezara viridula	0.4±0.24	Alfalfa	Nymphs	23/08/13, 65m
Nezara viridula	1.25±0.94	Alfalfa	Nymphs	30/08/13, 110m
Cercopidae	1.2±0.92	Weeds	Adults	24/03/13 90m
Eurydema ornatum	3.1 ± 0.9	Weeds	Adults	24/03/13 100m
Eurydema ornatum	8.1 ± 1.9	Weeds	Adults	12/04/13, 90m
Eurydema ornatum	4.3 ± 0.9	Weeds,	Nymphs	17/05/13, 100m
Eurydema ornatum	5.1 ± 1.2	Weeds	Nymphs	8/06/13, 410m
Eurydema ornatum	4.1 ± 0.9	Weeds	Nymphs	11/07/13,350m
Eurydema ornatum	6.2 ± 1.9	Alfalfa	Nymphs	26/07/13, 480m

Table 3. (Continued)

	Average number of nodules	Sources	Developmental stages	Collection dates, altitudes
Eurydema ornatum	5.9 ± 0.68	Weeds	Adults	16/08/13, 48m
Eurydema ornatum	0.66 ± 0.66	Weeds	Nymphs	30/08/13, 110m
Aelia rostrata	4.7 ± 2.8	Wheat	New generation adults	28/06/13, 600m
Cicadidae	6.2 ± 2.1	Weeds	Adults	5/07/13,100m
Rhyncoris sp.	11.3 ± 2.1	Weeds	Adults	14/06/13, 350m
Rhyncoris sp.	7.9 ± 1.9	Weeds	Adults	26/07/13, 410m
Dolycoris baccarum	13.2 ± 0.92	Weeds	Adults	5/04/13, 90m
Dolycoris baccarum	2.1 ± 1.1	Weeds	Nymphs	17/05/13, 400m
Dolycoris baccarum	2.1 ± 0.9	Weeds	Nymphs	31/05/13, 410m
Carpocoris mediterranus	21.2 ± 4.2	Weeds	Adults	17/05/13, 400m
Carpocoris mediterranus	28.32 ± 11.2	Weeds	Adults	8/06/13, 400m
Carpocoris mediterranus	6.2 ± 2,3	Weeds	Adults	30/08/13, 110m
Carpocoris sp.	7.24 ± 3.18	Alfalfa	Adults	11/07/13, 95m
Carpocoris sp.	8.46 ± 3.1	Alfalfa	Adults	2/08/13, 65m
Lygaeidae	18.2 ± 3.2	Soil	Adults	5/07/13,100m
Miridae	0.85 ± 0.34	Weeds	Adults	30/08/13,110m
Orthoptera				
Acrididae	11.3 ± 3.4	Weeds	Nymphs	19/04/13, 100m
Acrididae	9.42± 0.94	Weeds	Nymphs	3/05/13, 73m
Acrididae	20.33± 4.2	Weeds	Adults	31/05/13, 410m
Acrididae	22.3 ± 5.2	Weeds	Nymphs	8/06/13, 100m
Acrididae	31.2 ± 9.3	Weeds	Adults	11/07/13,350m
Acrididae	32.6 ± 6.26	Weeds	Adults	19/07/13, 65m
Acrididae	82.8 ± 11.2	Soil	Adults	2/08/13, 60m
Acrididae	52.8 ± 6.63	Alfalfa	Adults	16/08/13, 60m
Acrididae	57.12 ± 7.02	Alfalfa	Adults	23/08/13, 65m
Acrididae	56 ± 10.39	Soil	Adults	30/08/13, 110m
Poecilimon spp. (Tettigoniidae)	58.32 ± 7.2	Soil	Nymphs	5/04/13, 90m
Poecilimon spp. (Tettigoniidae)	38.2 ± 3.8	Weeds	Nymphs	26/04/13, 85m
Poecilimon spp. (Tettigoniidae)	6.55 ± 0.88	Weeds	Nymphs	3/05/13, 73m
Poecilimon spp. (Tettigoniidae)	30 ± 3	Weeds	Nymphs	10/05/13, 68m
Poecilimon spp. (Tettigoniidae)	32.3 ± 7.4	Weeds	Nymphs	8/06/13, 100m
Poecilimon spp. (Tettigoniidae)	31.2 ± 7.3	Weeds	Nymphs	28/06/13, 410m
Poecilimon spp. (Tettigoniidae)	22.6 ± 3.28	Weeds	Nymphs	30/08/13, 110m
Gryllotalpa gryllotalpa	67.3 ± 10.2	Soil	Adults	24/05/13, 90m
Diptera				
Eristalis arbustorum	19.5 ± 1.5	Weeds	Adults	18/02/13, 360m
Eristalis tenax	16.3 ± 1.7	Weeds	Adults	18/02/13, 361m
Eristalis tenax	11.8 ± 3.1	Weeds	Adults	24/03/13, 420m
Eristalis tenax	8.2 ± 1.3	Weeds	Adults	17/05/13, 400m
Eristalis tenax	7.1 ± 1.06	Weeds	Adults	21/06/13, 450m
Phasmida				
Phasmidae	6 ± 1.23	Weeds	Adults	21/01/13, 138m
Gratidia sp.	8.6 ± 2	Weeds	Nymphs	14/06/13, 350m
Gratidia sp.	13.8 ± 3.26	Weeds	Adults	11/07/13, 350m
Dermaptera				
Dermaptera	26.75 ± 1.49	Soil	Adults	18/02/13, 321m
Hymenoptera				
Cephus pygmaeus	3.9 ± 0.99	Wheat	Larvae	31/05/13, 410m
Apidae	8.1 ± 3.1	Weeds	Adults	21/06/13, 350m
Apis mellifera	4.9 ± 0.59	Weeds	Adults	28/06/13, 410m
Vespidae	4 ± 2	Weeds	Adults	30/08/13, 110m
Odanata				
Libellulidae	30.1 ± 2.8	Near the water	Adults	21/06/13, 350m
Libellulidae	30.5 ± 3.5	Near the water	Adults	19/07/13, 65m
Libellula depressa	8.5 ± 3.5	Near the water	Adults	30/08/13, 110m

Table 4. A single-factor ANOVA across species for collection sources differences

Year	Collection sources	Nodules /insect[a]	Number of individuals
2011	Plant material	18.3±8.5[a]	140
	Soil	24.65±3.8[a]	20
2012	Plant material	14.7±2.0[b]	740
	Soil	37.9±9.0[a]	40
2013	Plant material	15.5±1.6[b]	1370
	Soil	58.2±9.1[a]	50

[a]Mean number of nodules in a column followed by different letters are significantly different for each year {($F_{(1,14)}$ = 0.07, P = 0.7915 for 2011), ($F_{(1,76)}$ =6.72, P <0.05 for 2012) and ($F_{(1,140)}$ = 23.86, P < 0.0001 for 2013)}

Table 5. A single-factor ANOVA across species for developmental stage differences

Year	Developmental stages	Nodules /insect	Number of individuals
2011	Adults	23.0±12.1[a]	90
	Larvae	16.05±8.8[a]	60
	Nymphs	2.70±0.4[a]	10
2012	Adults	16.0±2.6[a]	560
	Larvae	15.9±3.0[a]	170
	Nymphs	15.44±7.6[a]	50
2013	Adults	16.2±2.1[a]	1060
	Larvae	24.2±3.1[a]	150
	Nymphs	14.2±3.4[a]	210

No significant differences were detected for each year{($F_{(2,13)}$ = 0.23, P=0.7957 for 2011), ($F_{(2,75)}$ = 0.00, P=0.9973 for 2012) and ($F_{(2,139)}$ = 1.19, P=0.3084 for 2013)}

Table 6. A single-factor ANOVA across species for insect order differences

Year	Insect orders	Nodules /insect[a]	Number of individuals
2011	Lepidoptera	16.0±8.8[a]	60
	Hemiptera	28.2±22.39[a]	50
	Coleoptera	6.8±4.3[a]	20
	Orthoptera	13.4±7.4[a]	20
2012	Lepidoptera	12.2±2.9[b]	180
	Hemiptera	13.2±5.1[b]	200
	Coleoptera	5.1±1.2[b]	180
	Orthoptera	33.2±3.7[a]	110
	Hymenoptera	9.2±3.3[b]	50
	Odanata	44.5±9.8[a]	40
2013	Lepidoptera	17.0±2.2b[a]	300
	Hemiptera	18.2±4.7b[a]	390
	Coleoptera	6.9±1.3b	380
	Orthoptera	36.7±5[a]	180
	Hymenoptera	5.2±0.9[b]	40
	Odanata	23±7.2b[a]	30
	Diptera	12.5±2.3[b]	50

[a]Mean number of nodules in a column followed by different letters are significantly different for each year {($F_{(3,11)}$ = 0.24, P=0.8634 for 2011), ($F_{(5,70)}$ = 7.93, P<0.0001 for 2012) and ($F_{(6,131)}$ = 5.33, P<0.0001 for 2013)

Table 7. A single-factor ANOVA across species for altitude differences

Year	Altitudes	Nodules /insect[a]	Number of individuals
2011	0-150 m	64.6±52.8[a]	20
	151- 300 m	12.6±4[b]	140
2012	0-150 m	25.0±10.8[a]	90
	151- 300 m	21.6±5.3[a]	170
	301-450m	12.8±1.6[a]	510
2013	0-150 m	17.6±2.3[a]	90
	151- 300 m	2.1±0.4[a]	10
	301-450m	16.1±2.6[a]	430
	450m- …	10.1±4.6[a]	80

[a]Mean number of nodules in a column followed by different letters are significantly different for each year {($F_{(1,14)}$ = 7.62, P<0.05 for 2011), ($F_{(2,74)}$ = 3.05, P=0.0536 for 2012) and ($F_{(3,138)}$ = 0.51, P=0.6771 for 2013)}

Discussion

Insects pysiologically produce two categories of defense responses to microbial infections, humoral and hemocytic defence reactions (Dunn, 1986; Gupta, 1991). Humoral reactions take several hours for their full expression, and involve induced synthesis of antibacterial proteins, such as cecropins, attacins, diptericins, and defensins (Dunn, 1986). In the presence of these proteins, bacteria lose their cellular integrity because of the detergent properties of peptides. Insects also synthesize lysozymes, which enzymatically attack bacteria by hydrolyzing their peptidoglycan cell walls (Dunn, 1986; Russell & Dunn, 1996).

Hemocytic reactions involve direct cellular interactions between circulating hemocytes and bacteria. In contrast to humoral defense reactions, hemocytic responses are very quick, typically occur within minutes of an infection cycle. Specific cellular defense mechanisms include phagocytosis, nodulation and encapsulation (Gupta, 1991).

Nodulation reaction is one of insect cellular or hemocytic defense actions. Dunn and Drake (1983) indicated that following an injection of bacterial cells into tobacco hornworm *Manduca sexta*, the insects were capable to clear most bacterial cells from their hemolymph circulation by nodulation in the first 2 h following the artificial infection. Occurence of nodulation involves more than one steps. First, insect granulocytes attache to infecting microbe cells, second, the granulocytes are degranuted that causes attraction of insect plasmatocytes to the growing nodule, and the spreading of plasmatocytes around the nodule (Dean et al., 2004). Finally, the darkened, melanized nodules attach to an internal organ or body wall, where they remain through the life of the insect.

Nodules are not easilly moved away from insect hemocoels when insect has experienced a microbial infection. The presence of nodules in insect hemocoels indicates that the insect was infected with microbes in the past or has a past microbial infection. Many researchers report nodulation reactions in insects following infections of insects with microbes including bacteria, fungal spores and some viruses (Miller et al., 1994; Dean et al., 2002; Lord et al., 2002; Büyükgüzel et al., 2007; Durmuş et al., 2008). Howard et al. (1998) also reported that some bacterial species evoked far more nodules than similar infections with other species.

In this paper, we obtained results which support the hypothesis that insects mostly have experienced microbial infections in nature, they recovered and continued living. The results of all experiments support this hypothesis. Nodules were seen in virtually all examined insect specimens, altough we recorded various numbers of nodules in different insect specimens, which indicate that depending on conditions, insect may have small number of invaders or large number of invaders in nature. Moreover, when we tested major insect orders, including Coleoptera, Lepidoptera, Hemiptera, and Orthoptera for nodulation, the nodules were seen in all tested specimens from which we infer the finding that apply to most insect species. Finally more nodules were seen in the insects collected from soil, a site of significant microbial challenge, than in the insect collected from other sites. These results showed that insects may face significant microbial infections during their lives but survive because their immune systems are capable of overcoming the microbial infections in nature.

As referred above, far more nodules observed in insects associated with soil than in insects collected from plant materials, such as the orthopterans and sunn pest adults. This result is important because previous studies showed that using imidacloprid and entomopathogenic fungi together will lead to increasing mortality of soil pests (Boucias et al., 1996; Quintella & McCoy, 1997). The result also showed that overwintered insect generations, *Ostrinia nubilalis, Sesamia nonagrioides* and *Eurygaster integriceps,* had much more nodules as compared to new generation larvae of *O. nubilalis, S. nonagrioides* and adults of *E. integriceps.* Similarly, Tunaz & Stanley (2009) and Tunaz et al. (2015) reported that the new generation of sunn pests had very few nodules as compared to older, overwintered adults collected from Kahramanmaraş and Adana provinces of Turkey. We recorded no nodulation number differences at different developmental stages and altitutes of insects collected. However, in 2011, the insects collected at lower altitudes showed more nodules than the insects collected at higher altitudes Our data also indicated that significantly more nodules were seen in the orthopteran species than in the lepidopteran, hemipteran, and coleopteran species, which is reasonable since orthopteran species were mostly collected from soil, which support the findings of Tunaz & Stanley (2009) and Tunaz et al. (2015). Drawing together, main issues that cause higher numbers of nodules in insects are insect orders and soil contact of insects. From these results, we can speculate that all insects are exposed to possible infection, and the actual occurrence of a natural infection is a random event. Collected specimens in our study were healty in the field. These observations mean that the insects had been infected by microbes, and by the time of our collections they had either checked the invasion or had recovered from the infections.

Pests cause crop losses about 30–40% per year, depending on the particular crop, a large proportion of which is due to insects (Oerke & Dehne, 2004). Therefore understanding of insect immunity and pathogens on insect immunity are very important for controlling pest insects in agriculture, in which microbial insecticides are used. Insects have the ability to recover from infections in nature which is important for biological and agricultural implications. On the other hand, biologically, many microbes have the ability to overcome insect immune systems. Wang & St. Leger (2006) reported that the fungal insect pathogen, *Metarhizium anisopliae* produces a 60.4-kDa gene product, which effectively hides the hyphal bodies from immune surveillance of insects. Similarly, Stanley & Miller (2006) indicated that the bacterium *Xenorhabdus nematophila* secretes factors that inhibit the eicosanoid signaling, which is crucial to launching cellular immune reactions. Therefore, we need to understand evolutionary mechanisms of insect immunity and inhibitory actions of insect pathogens for getting effective result on insect pest management. It is known that insect immunity sytems may limit use of biopesticides, which are environmentally friendly insecticide. Hence, in this paper, we tried to understand insect immune reactions in nature in which the insects were collected from Hatay Province of Turkey.

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