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ORIGINAL ARTICLE

Melanin properties at the different stages towards life cycle of the fly *Hermetia illucens*

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Eumelanin type pigments are synthesized at all the stages of the life cycle of the fly *Hermetia illucens*: in the larvae, pre-pupae, pupae and adult flies (dead flies). The greatest content of melanin was recorded in the cuticles. Melanin was present not only in the cuticle, hence it remained in the cuticle after the emergence of the adult fly. It was also found in the insect body in a complex with lipids. In pupae, it is mostly lauric acid that was associated with melanin. Its proportion in the melanin-chitosan complex was 80%. The isolated melanin-chitosan complex of adult flies showed a wide range of antibacterial activity, inhibiting the growth of 21 out of the 25 of the test cultures. The melanin-chitosan complex of empty pupal membranes and alcohol suspension of pupal melanin inhibited twice as smaller number of test cultures and the above activity was absolutely in the pupal chitosan. The largest zone of growth inhibition was recorded with respect to Aspergillus niger, Candida albicans, salmonella, and Staphylococcus aureus. An alcohol suspension of pupal melanin inhibited the growth of 10 test cultures. In this case the greatest activity was shown in relation to Mycobacterium B5 and Acinetobacter sp. 1182.
Key words: melanin; melanin-chitosan complex; *Hermetia illucens;* antibacterial activity

The fly *Hermetia illucens* is a typical member of Stratiomyidae, Diptera, Insecta (Linnaeus, 1758) is characterized by complete metamorphosis, including the main developmental stages as the egg, larva, pre-pupae, pupae, adult individual. It is only larvae that feed. Larvae are able to recycle different organic waste, using them as foraging substrates. This property determines the increased interest in these insects and the peculiarities of their biochemical composition in connection with the prospects of using insect meal in feed and food (. Although the chemical composition of the insect larvae varies with food substrate the fat content averages 30% (Diener, et al., 2011; St-Hilaire, et al., 2007; Gobbi, et al., 2013; Kalová, Borkovcová, 2013). Adult flies are equally fat. Our data demonstrate that fat content in dead flies accounts for 27%, and that in the larvae raised on wheat grains, 38%, and in prepupae, 32%. The large amount of fat in the body of the fly necessitates its antioxidant protection, which may be provided by melanin pigments. Melanins are nitrogen-containing compounds of irregular structure on the basis of phenol, which are the main pigment of insects. They create the dark-brown or brown coloration of insects, being created by the insects themselves, and become part of the cuticle, are present in the hemolymph, adipose body, and the intestinal tract (Nicolaus, 1968; Prota, 1992). The pigments have antioxidant, radio- and photo-protector, electron and ion-exchange properties (Dontsov, 2014; Ostrovsky, Dontsov, 1985). Natural melanin pigments show antibacterial and immune-modulation activity. They can form diverse complexes with proteins, various pharmacological agents, for instance, with antibiotics, which indicates a wide range of melanin biological activity (Montefoiori, Zhou, 1991; Martikyan, et al., 2015; Meredith, Sarna, 2006).

The body of an adult *Hermetia illucens* is black in color, which is reflected in the name of the fly. The larvae are light-brown in color and pre-pupae are dark-brown. The latter indicates a considerable amount of melanin in *H. Illucens.* However, data on the structure and functional properties of those pigments in in the insect concerned are virtually lacking. The objective of the present study is obtaining and identification of melanin of different age stages in *H. illucens*: larvae, larvae before pupation (pre-pupae), dead flies and the study of some of their properties.

Material and methods

The study subject was the fly *H. Illucens*. The larvae were raised in the laboratory of the Institute of the Problems of Ecology and Evolution, RAS, using wheat grains as food substrate.
Melanin was obtained from the biomass of dead flies, dry larvae, pre-pupae, pupae before the emergence of imago, and empty pupal membranes that remained upon emergence of the flies by the method (Pogarskaya, et al., 2008). Pre-dried biological material was ground in a mortar to reach the particle size of no more than 3-4 mm. Subsequently, the powder obtained was added 10% NaOH («Sigma», USA) at a ratio of 1:10 of mass to be deproteinated to isolate chitin when mixing repeatedly at t =80°C in a closed vessel without access of the air for 1.5 hours. Subsequently, upon cooling the same vessel was added the same amount of 50% NaOH and chitin desacetylation was performed in a closed vessel without access of the air at t =95°C for 2.0 hours, stirring repeatedly. The hydrolysate obtained was filtered in the Buchner funnel. The residue (chitosan) was washed to reach a neutral reaction of rinse waters (pH 7). Melanins were removed from alkaline hydrolisate by sedimentation with concentrated hydrochloric acid to pH 2 and subsequent centrifugation at 1500 g for 15 minutes (centrifuge Beckman Allegra 64R). The isolated residue was washed to reach a neutral reaction of rinse waters or dried up at t=50°C to constant weight or added to the previously isolated chitosan to obtain a melanin-chitosan complex, which was dried up also at a temperature of 50°C.
When the melanin residue of the pupae was centrifuged, it was divided into two fractions – the residue proper and the hydrophobic fraction over the supernatant (a complex of melanin and fat). Upon separation of the residue, a separation funnel was used to isolate a complex of melanin and fat. The isolated fractions were dried up as described above.
Pigment identification was based on assessment of their solubility, spectral and paramagnetic properties (Ostrovsky, Dontsov, 1985; Bilińska, et al., 2002.). Measurements of the spectra of the electron paramagnetic resonance (EPR) were performed in the EPR spectrometer Bruker EMX (Germany). The registration of the spectra was performed for dry specimens of 50 to 200 mg placed in a cylindrical quartz cuvette. The conditions of EPR spectra recording were as follows: modulation amplitude 1.25-3.0 Gc, sweep range 50 Gc, microwave frequency, 9.8 GGc , SHF, 0.2 мWt, time constant, 100 msec. To estimate g-factors and determine spin concentration in melanin samples the standard sample UDA №5 was used, calibration certificate № 905/910-2012. The content of melanin in the sample under study was estimated by comparison of the quantity of the pure DOPA-melanin not containing protein admixtures, whose concentration of paramagnetic centers determined under the same conditions was 1.94×10^{18} spin/g of dry weight. DOPA-melanin was obtained in the course of oxidative polymerization of dioxyphenylalanine in low alkaline medium and purified by the method (Porebska-Budny, et al., 1992). To estimate the melanin content in an individual it was assumed that the mean mass of dry body was: larva – 79 mg, pre-pupa - 72 mg, pupa – 65 mg, pupal membrane - from a pupa – 15 mg, adult fly (dead fly) – 11 mg.
Extraction of lipids from `the melanin complex and fat of the pupae (0.35 g) was performed in a glass ampule with a screw cap by addition of a 3 ml of mixture DCM-methanol (10:1 volume) and treating in a US-bath for 15 minutes The extract was silylated by BSTFA and analyzed in a gas chromatograph Finnigan Trace GC Ultra with a capillary column DB-5ms 25m-0.25mm-0.25µ) and mass spectrometric detector Finnigan Polaris Q. The sample was introduced in a splitless mode with purging of the injector after 0.1 minutes. The initial temperature of the thermostate was 60°C for 2 minutes, then temperature rose to 300°C at a rate of 6°C/min and hold for 6 minutes. The injector temperature was 250°C, ion source 220°C, interface 250°C. The carrier gas helium flow was 1 ml/min in a constant flow mode. The mass spectra were recorded at the range 41-550 Da. Identification was performed on the base of spectrostructuralcorrelations and library search. The content of acids was estimated by internal normalization of the total ion current (TIC) chromatographic peaks area, assuming that responce factors for all the compounds are equal.
The antagonistic activity was determined by the method of agar diffusion (Egorov, 1994.) in relation to test cultures of microorganisms from the collection of the Institute of Microbiology and Virology (Almaty). To determine the antagonistic activity suspensions were prepared from melanin, chitosan or melanin-chitosan complexes in a sterile aquatic solution, and, in addition, suspensions were prepared from melanin or chitosan in acidified ethyl alcohol (alcohol with 0.5% HCl), at 10 mg/ml, which were applied on the surface of solid nutritional medium in Petri dishes inoculated with test cultures. The incubation was at 35°C for 24 hours. The antagonist activity was deduced from the zones of growth of the test culture around the applied specimen. For the alcohol suspension of melanin or chitin, the antagonistic activity was defined as a difference between the zone suppression of the suspension concerned and the control (acidified alcohol). The diameter of the suppression zone was estimated with a trammel «Vernier caliper 0-150 mm» «Mitutoyo 530-101» (Japan) to a precision of 0.05 mm. For mathematical treatment of the results standard methods for obtaining mean values and their mean errors were applied.

Results

The melanin-like pigment was isolated from all the specimens of biomass of different stages of the life cycle of the fly *H. illucens*.
Pigment was obtained from dead flies (specimen №1), which according to the parameters of the EPR signal corresponded to eumelanin: g - factor -2.0036; ΔH – 5.6 ± 0.2 Gs, which is close to DOPA-melanin, whose line width (ΔH) is 4.0 ± 0.5 Gc and the value of the g factor of 2.0034 ± 0.0006 (Enochs, et al., 1993). The concentration of the paramagnetic centers in specimen №1 – 5.6×10^{16} spin/gram of dry weight, which corresponded to 29 mg of per 1 g of the dry mass of dead flies.

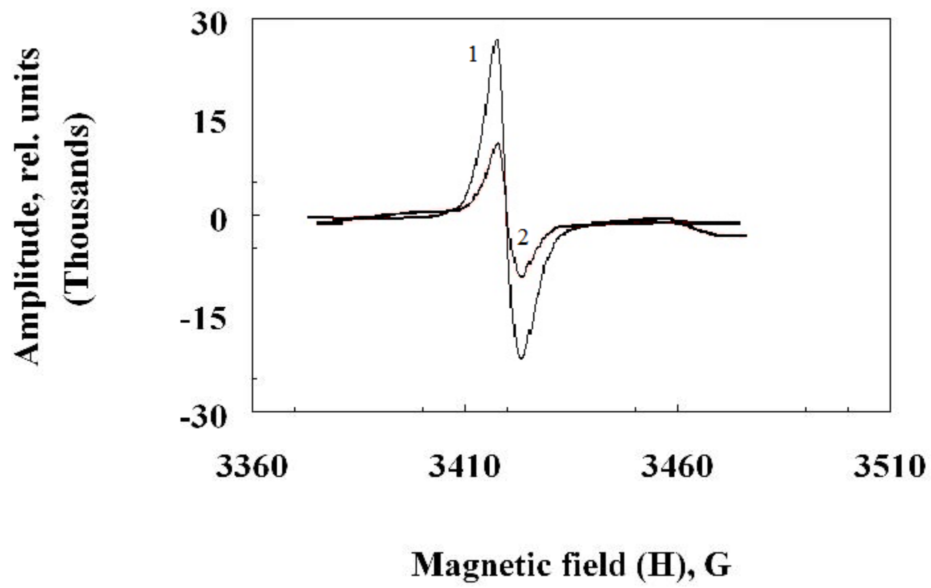

Fig. 1A

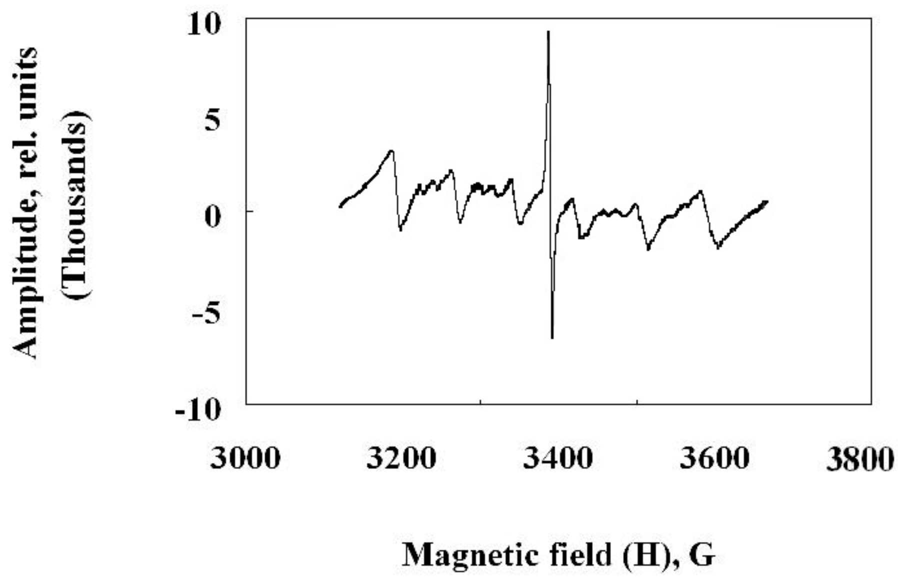

Fig. 1B

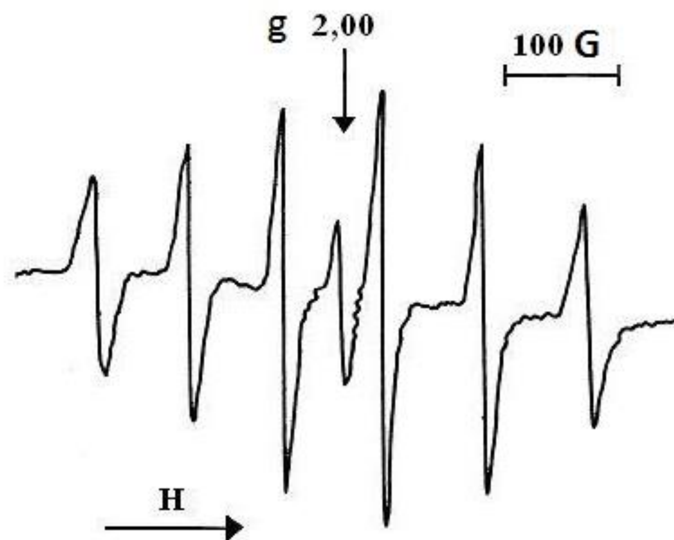

Fig. 1C

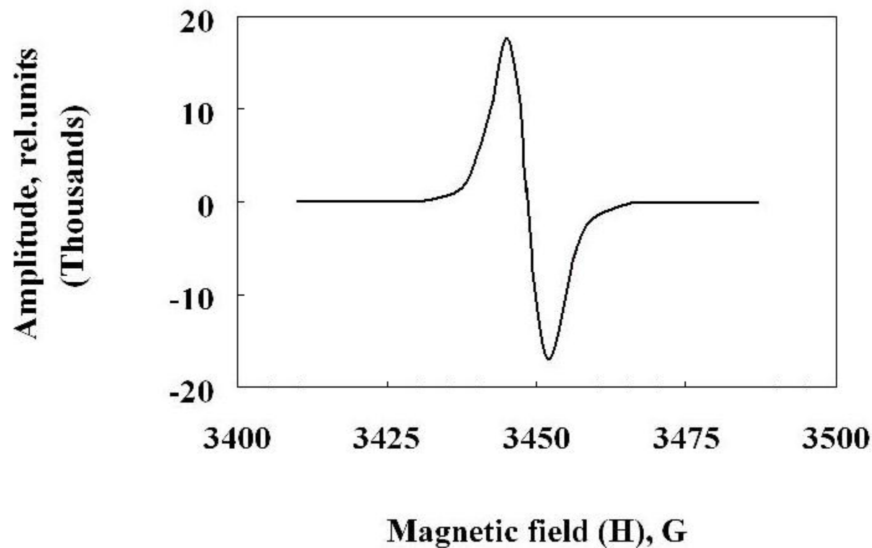

Fig. 1D

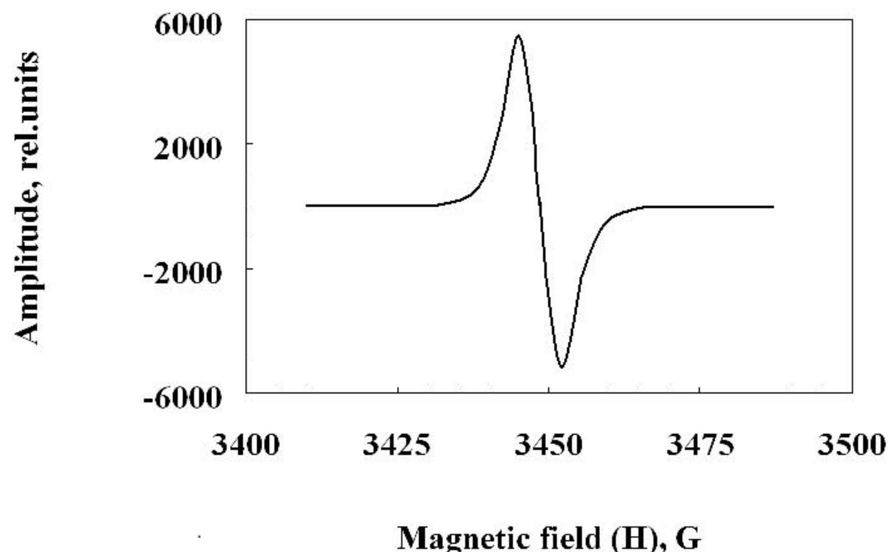

Fig. 1E

Fig. 1. EPR spectra A – combined spectra 1- of specimen №4 (pupa) and 2 – specimen №3 (prepupa); B – specimen №3 (prepupa) with an expanded range of the scanning of the magnetic field; C – bivalent manganese; D – specimen №5 melanin of pupae in sediment); E – specimen №6 (the melanin-lipid complex of pupae).

The EPR signal of the pigment of dry fly larvae (specimen №2) was slightly asymmetric with g factor equal to 2.0036 and the ΔH value equal to 6.1 ± 0.7 Gs. Since the EPR eumelanin signal has the line widths within the range of 4 to 6 Gs depending on the nature of melanin (Meredith, Sarna, 2006), there is reason to believe that the pigment from the larvae with a characteristic singlet EPR signal with a line width of 6.1 Gs, belongs to the class of eumelanins. The concentration of the paramagnetic centers in specimen №2 was 1.1×10^{16} spin/g dry weight, which is almost 5 times as little as that for dead flies and corresponded to 5.7 mg of melanin per 1 g of dry mass of the larvae.

Specimen №3 is melanin from prepupae (Fig. 1A, B): ΔH – 5.7 Gs, g-factor – 2.0036, the spin concentration is 8×10^{16} spin /g of the specimen dry weight, which corresponded to 32 mg of melanin /g of dry weight of the pre-pupae. The nature of the signal indicated that in addition to melanin the specimen contained some other paramagnetic substances (presumably, bivalent manganese).

Specimen №4 is melanin from the pupae (Fig. 1A). Its characteristics are as follows: ΔH – 5,8 Gs, g-factor – 2.0037, spin concentration – 12.4×10^{16} spin/g specimen dry weight, which corresponded to 34 mg of melanin/g of the dry weight of the pupae. The EPR of the pupal melanin signal is more pronounced compared with the signal of the specimen from the prepupae and it had no signal of supposedly bivalent manganese. Fig. 1A shows a comparison of two EPR spectra – pupal samples (curve №1) and pre-pupae (curve №2) with a limited range of the sweep of the magnetic field 3380 to 3480 Gs. The prepupa specimen showed some characteristic rises and declines of the signal, which become more pronounced as the sweep range widens as shown in Fig. 1B. The spectra concerned had some features in common with the EPR range of the bivalent manganese (Fig. 1B).

The EPR Mn^{+2} range is a typical six-band range, with 3 bands left of the center and 3 right of the. In the presence of melanin, the signal in the center is higher than in the range of pure manganese and the bands are not so well-defined, which may be associated with the superposition of the melanin signal.

Specimen №5 is melanin from the membrane of the pupae after the emergence of the pupae from the newborn fly. This signal is also typical of eumelanin. The ΔH value and the shape of the signal indicated the eumelanin type of pigment. The concentration of paramagnetic centers in that specimen was $3.7x10^{16}$ spin/g dry weight, which corresponded to the content of 19 mg of melanin per 1 g of dry membranes of the pupae.

Out of 15038 mg of dry pupae 60 mg of melanin-containing precipitate was obtained (specimen №6) and 410 mg of the complex of melanin and fat (specimen №7). Fig. 1B shows the EPR of the range of specimen №6, representing eumelanin almost free from admixtures. The concentration of paramagnetic centers in this specimen is $1.1x10^{18}$ spin/g of dry weight, g-factor being 2.0028. The content of pure melanin in that specimen corresponded to 570 mg/g of dry weight, which in relation to 60 g of precipitate was 34.2 mg. Specimen №7 was also eumelanin (Fig.1D). The concentration of the paramagnetic centers in that specimen was $1.3x10^{17}$ spin/g of dry weight, g-factor being 2.0025. The melanin content in specimen №6 corresponded to 67 mg/g of dry weight, which accounted for 27.5 mg in 410 mg of the melanin-lipid complex. The total amount of melanin in 15038 mg of dry pupae is 61.7 mg or about 0.4%, out of which amount 0.23% precipitated and 0.18% was extracted with fat.

Table 1. Parameters of EPR signals of melanin pigments in the biomass of the different stages of the life cycle of the fly *H. illucens*

Specimen №	Biomass source	g – factor	ΔH, Gs	Concentration of paramagnetic centers, spin/g, dry weight of the specimen	mg melanin/g dry mass, individual stage of *H. illucens*	mg melanin/ individual
1	Dead flies	2.0036	5.6±0.2	$5.6x10^{16}$.	29.0± 3.5	0.32± 0.04
2	Dry larvae	2.0036	6.1 ± 0.7	$1.1x10^{16}$	5.7 ± 0.7	0.45 ± 0.06
3	Dry prepupae	2.0036	5.7±0.5	$8.0x10^{16}$	22.0 ± 3.3	1.58 ± 0.24
4	Dry pupae	2.0037	5.8±0.5	$1.24x10^{17}$	34.0 ± 4.0	2.21 ± 0.26
5	Membranes of pupae	-	5.8 ± 0.4	$3.7x10^{16}$	19.0 ± 2.2	0.28 ± 0.03

Table 2. Composition of the lipids of the melanin-lipid complex of the larvae *H. illucens*

RT, min	m/z	Compound	Concentration, % rel.
15.89	75;229;247	Decanoic acid	0.8
17.28	74;87;171;215	Methyl ether of dodecanoic acids	0.9
19.88	257;275	Dodecanoic acid	80.5
21.09	74;143;199	Methyl ether of tetradecanoic acid	0.1
23.00	117;283;301	Tetradecenoic acid	0.1
23.25	117;285;303	Tetradecanoic acid	1.9
24.17	67;236	Methyl ether of hexaethyl of hexadecenic acid	0.1
24.39	117;299;317	Pentadecanoic acid	0.1
24.54	74;143;227;270	Methyl ether of hexadecanoic acid	0.2
26.10	117;311;329	Hexadecenic acid	1.7
26.46	117;314;331	Hexadecanoic acid	2.5
27.16	67;262	Methyl ether of 6,9-octadecanoic acid	0.2
27.27	67;264	Methyl ether of 9-octadecadienic acid	0.4
28.91	178;262;337	9,12- octadecadienic acid	2.6
29.04	117;340;357	9- Octadecenic acid (oleic)	0.1
29.41	117;341;359	Octadecanoic acid a	0.9

Table 1 presents some summary data on the parameters of the EPR signals of melanin pigments in the biomass and in an individual at different stages of the life cycle of the fly *H. illucens*. The largest content of melanin in dry biomass was revealed in the pupae (34 mg of melanin per 1 g of dry biomass), similar to the quantity of melanin per one pupa (2.21 mg/individual). At the pre-pupa stage there was intensive pigment synthesis. The melanin content in the pre-pupa was 1.58 mg/individual, which was almost three times as high as the melanin level in the larva (0.45 mg/individual). An empty membrane of the pupa upon emergence of the contained the least melanin quantity (0.28 mg/individual). The imago contained less melanin than the larva (0.32 mg/individual), however, the total quantity of pigment per 1 g. of dry dead flies was 29 mg, which is comparable to the respective indices for the pupae. Changes in the content of melanin in the biomass from different stages of the fly life cycle are shown in Table 2.
It has been revealed the melanin of the pupae can be isolated not only in the pure form but also jointly with lipids. The bulk of the lipids in a complex with melanin were fatty acids (Table 2). Lauric (dodecanoic) acid predominated, its content accounting for 80 %. Saturated acids (C10 – C18) were present in small amount, as well as monounsaturated acids C14, C16, C18 and unsaturated acid C18, and also methyl ethers of the C12-C18.
The dead flies, pupae, membranes of the pupae provided melanin-chitosan complexes and also melanin or chitosan that were studied for antimicrobial activity. The results obtained are presented in Table 3.

Table 3. The antimicrobial activity of melanin and melanin-chitosan complexes of *H. illucens* (zones of inhibition of growth of the test cultures, mm)

Test cultures	Melanin-chitosan complex		Melanin suspension in ethanol
	Dead flies	Membranes of pupae	Pupae
Escherichia coli-K	0	0	0
Salmonella gallinarum	12.0±0.3	0	0
Staphylococcus aureus MRSA 3316	12.0±0.3	0	12,5±0,3
Staphylococcus aureus MRSA 9	9.0±0.2	0	12±0.3
Klebsiella pneumoniae 444	12.0±0.3	0	12±0.3
Candida albicans	12.0±0.3	нет	11±0.2
Pasteurella multocida	12.0±0.3	9.0±0.2	11±0.2
Aspergillus niger	15.0±0.5	0	0
Proteus sp.	9.0±0.2	0	0
Bacillus subtilis	12.0±0.3	0	0
Pseudomonas aeruginosa 342	0	0	0
Pseudomonas aeruginosa 835	9.0±0.2	0	0
Acinetobacter sp. 1182	0	0	16±0.6
Acinetobacter sp. 1522	9.0±0.2	0	11±0.2
Yersinia pestis 1m	12.0±0.3	9.0±0.2	12±0.3
Staphylococcus aureus (VISA) 2m	12.0±0.3	15.0±0.5	0
Staphylococcus hyicus 3m	12.0±0.3	12.0±0.5	0
Staphylococcus aureus (VISA) 4m	12. ±0.3	9.0±0.2	10±0.2
Citrobacter freundii complex 6m	9.0±0.2	0	0
Serratia odorifera 7m	9.0±0.2	0	0
Cedecia sp. 8m	12.0±0.3	9.0±0.2	0
Kluyvera ascorbata 9c	9.0±0.2	9.0±0.2	0
Klebsiella oxytoca 10c	0	0	0
Enterobacter intemedius 11c	9.0±0.2	0	0
Serratia liguefaeciens 12c	9.0±0.2	0	0
Mycobacterium B5	0	0	16,5±0.6

The melanin-chitosan complex from dead flies showed a wider range of antimicrobial activity as compared with the respective complex from the membranes of the pupae, pupae and the melanin of the pupae (alcohol melanin suspension). The melanin-chitosan complex failed to inhibit the growth of only 4 test cultures out of the 25 tested. The highest antagonistic activity in that specimen was revealed in relation to the fungus *Aspergillus niger* (the zone of growth inhibition is 15 mm), and also in relation to the yeast *Candida albicans,* intestinal bacteria *Salmonella gallinarum,* conventional pathogens *Staphylococcus aureus* 3316, *Klebsiella pneumoniae* 444, *Pasteurella multocida, Bacillus subtilis, Yersinia pestis* 1m, *Staphylococcus aureus* (VISA) 2m, *S. hyicus* 3m, *S. aureus* (VISA) 4m, *Cedecia sp*. 8m (the zone of inhibition of test cultures 12 mm). With regard to the other test cultures of the zone of inhibition of growth were 9 mm.

The melanin-chitosan complex from the membranes of the pupae inhibited the growth of seven test cultures: *Pasteurella multocida, Yersinia pestis* 1m, *S. aureus*(VISA) 2m, *S.hyicus* 3m, *S. aureus* (VISA) 4m, *Cedecia sp*. 8m и *Kluyvera ascorbata* 9c. In this case, the greatest activity was revealed in relation to the staphylococci *S. aureus* (VISA) 2m (the zone of growth inhibition 15 mm), *S. hyicus* 3m (the zone of growth inhibition 12 mm).

As exemplified by pupae comparison was made of the antibacterial activity of chitosan and melanin. It was revealed that chitosan was antagonistic to neither of any of the tested cultures. Melanin from pupa in aquatic suspension was not antagonistic to any of the test cultures. The melanin from pupae proved antagonistic only in relation to *Mycobacterium* B5 and *Acinetobacter sp*. 1522. A wider range of the action of the alcohol melanin suspension was recorded. The suspension inhibited the growth of 10 test cultures from the 26 used for the experiment, including: *S. aureus* MRSA 3316, *S. aureus* MRSA 9, *S. aureus*(VISA) 4m, *Klebsiella pneumoniae* 444, *Candida albicans, Pasteurella multocida, Mycobacterium* B5, *Acinetobacter sp*. 1182 и *A. sp*. 1522, *Yersinia pestis* 1m. Melanin showed the highest activity in relation to *Mycobacterium* B5 and *Acinetobacter sp*. 1182. The zones of growth inhibition of those test cultures were 16.5-16.0 mm, crespectively, and those of the other test cultures concerned, 10-12.5 mm.

Discussion

The melanin pigments of animal origin fall into two major groups: eumelanins and phaeomelanins, whose EPR spectra differ considerably in terms of shape and parameters. The latter makes it possible to determine the melanin type by the shape of EPR spectrum (Montefoiori, Zhou, 1991). The present study demonstrates that the eumelamin type pigments are synthesized at all the stages of *H. illucens* life cycle: prepupae, pupae, adult flies (dead flies), but in different quantities. The greatest quantity of melanin was found in the pupae, At the prepupae stage intensive synthesis of pigment occurred. The empty membrane of the pupa contained the smallest quantity of melanin. In the imagines, the quantity of melanin was somewhat smaller per individual compared with that in the larva, however, the total quantity of pigment per of dead flies is comparable to that for pupae.

Melanin was present not only in the cuticle (and hence remained in the membrane of the pupae upon emergence of the adult fly) but also remained in the body of the insect in a complex with lipids, which is determined by the structural and functional properties of melanin, which are associated with its capacity to form complexes with electrophilic substances. As exemplified by pupae of *H. illucens*, it has been revealed that associated with melanin is essentially the lauric acid whose proportion in the lipid portion of the melanin accounted for 80%. The mass proportion of melanin in the complex did not exceed 7%, which proved to be almost a half of the entire melanin of the pupa. It is the presence of melanin that caused isolation of the lauric acid as a complex with pigment in case of alkaline hydrolysis, and the hydrophobic properties of the fatty acid determined the fluctuation of the complex, and the hydrolysis and the hydrophobic properties of the fatty acid also determined the fluctuation of the complex and the upper portion of the reaction mixture in case of acid sedimentation of the cuticle melanin. Thus, melanin in the cuticle was in two forms: half of the pigment was closely associated with the cuticle whereas the other half forms a complex with the body lipids. In this case, melanin as an antioxidant may not only perform the function of protection from oxidation but also promote the anti-infection function of insect protection.

It has been revealed that the prepupae of *H. illucens* contain substances with characteristics paramagnetic properties with a limited range of magnetic field sweep and, presumably, bivalent manganese. The above phenomenon requires a more detailed study. The larvae of pupae and imagines revealed no EPR signal similar to Mn^{+2} signal. Presumably, different stages of H. illucens cycle contain different paramagnetic substances. That may be associated with some internal biochemical rearrangements, including those involving intensive melanin synthesis.

Thus, the life cycle stages of the fly *H. illucens* under study are characterized by the presence of different forms of eumelanin capable of forming complexes with lipids and having antibacterial properties.

The isolated melanin-chitosan complex of adult flies showed a wide range of antibacterial activity. Twice as fewer number of test cultures inhibited the melanin-chitosan complex of empty membranes of pupae and the alcohol suspension of the melanin of pupae. Growth was most inhibited in case of absolute *Aspergillus niger*, *Candida albicans,* the intestinal bacteria *Salmonella gallinarum,* the conventional pathogens *Staphylococcus aureus*, *S. hyicus*, *Klebsiella pneumoniae*, *Pasteurella multocida, Bacillus subtilis, Yersinia pestis*, *Cedecia sp*. There are reasons to believe that the biologically-active melanin of an adult fly differs from that of pupae and the melanin of the membranes that remained empty after the emergence of the imagines of the membranes of the pupae, whose melanin-chitosan complexes showed a lower anti-infection properties. Presumably, the antibacterial activity of the melanin-chitosan complex is essentially a function of melanin content, which is supported by the absence of the activity concerned in chitin. The results obtained in the present study do not contradict published data available. It is shown that the antibacterial effect has methanolic extract larvae *H. illucens* (Choi W. H. et al., 2012). Our studies have shown that the antibacterial activity has melanin suspension in ethanol, which, apparently, due to the solubility of the melanin. Melanin is known to show bacteriostatic and antiviral effect (Montefoiori, Zhou, 1991; Martikyan, et al., 2015). It is well-known that the physico-chemical properties of eumelanin are essentially a function of its polymerization. In fact, it has been demonstrated that highly-molecular eumelanins show a by far higher antioxidant activity compared with the low-molecular products of their destruction (Zareba et al., 2006). In this connection, there are essential grounds to believe that eumelanins black vs. brown in color differ in terms of polymer structure. Since prepupae and pupae are brown in color whereas imagines are black, one can assume the presence of eumelanin- different in structure in different stages of the life cycle of a black larva, which may account for a wide range of antibacterial and antifungal activity in the black eumelanin of the imago of *H. illucens* as compared with the brown eumelanin of the pupae. Thus, the stages of the life cycle of the fly *H. illucens* are characterized by the presence of different forms of eumelanin capable of forming complexes with lipids and having antibacterial properties.

The revealed features of *H. illucens* melanins indicate new properties of black soldier fly biomass associated with the biological efficacy of using *H. illucens* larvae in animal nutrition. In previous studies, we found that the use of *H. illucens* fly in calves' feeds at a dose of 0.5 kg / t of mixed fodder improved metabolism, increased the indices of nonspecific immunity and normalized the microbiocenosis of the intestine of calves and increased average daily gain by 13.9% ($p<0.05$) in comparison with the control (Zelenchenkova, et al., 2017). Also in the physiological experiment (Nekrasov, et al., 2015) we have shown the possibility of a successful introduction in the diet of pigs 7% dried larvae *H. illucens* as an alternative to fish meal (5%). Also we used ultrasmall doses of the homogenate of raw insect (untreated hemolymph) in the composition with complex probiotic preparation. The results of study showed that the usage of dried larvae *H. illucens* in fodder increased average daily gain on 6.7-14.4% ($p>0.05$) as compared with controls.

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