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Periodic Research

Effects of Wound and Antibiotics on Blood Cells (Thcanddhc)of Dysdercus Koenigii

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Abstract

Six types of haemocytes are recognized in the present insect viz. Prohaemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Adipohaemocytes (ADs), Oenocytes (OEs), and Vermicytes (VEs). The haemocytes parameter i.e. total haemocyt count (THC) and differential haemocyte count (DHC) are reveals many fact related to defense and other physiological processes. Lower THC are found in insect group (have wound without application of antibiotics) then to their respective controls (wound with application of antibiotics). In comparison to insects group i.e. wound with antibiotics initially shows lower THC to their control (without wound and antibiotics) but in latter stage no significant differences observed. DHC study reveals higher PLs, GRs, OEs, and marginal VEs in insects group (wound without antibiotic) to their control. Normally PRs, ADs, PLs, are more in population in the controls than to their experimental group. Increasing trends of THC, PLs, PRs, and ADs, are observed in female adult insects before 1st egg cycle in both control and experimental. THC and DHC trends not shows any significant differences between male and female in both group (control and experimental).

Keywords: THC, DHC, Defense, wound, antibiotics

Introduction

The body cavity (haemocoel) of insect haemolymph (blood) flows with blood corpusceles (haemocytes). These haemocytes shows their involvement in various functions for examples phenolxidase activity, transport of nutrients, egg maturation, defense, intermediary metabolism, gene regulation, moulting, blood coagulation and connecting tissue formation (Berger et al 2003, Kurucz et al 2003, Mishra and Tiwari 2005, Merchant et al 2008, Greeny et al 2012, Pandey and Tiwari 2012). No doubt haemocytes are playing significant roles in insect life; besides this insect is the most diverse group of animals and shows great power of adaptation in various conditions either normal or extreme stressful. Because of these facts number of microbiologist, entomologist, zoologist, and biotechnologist are interested to investigate different aspects of insect's life. In them works on haemocytes are more. Most of the works related to recognition of haemocytes types, their ultra structure. No doubt number of information available on THC and DHC but mostly they are related to egg maturation, postembryonic studies, effects of some stress and nervectomy. In daily life like vertibrates, insect also suffering from simple and severe wounds and they heal and sustain with their immune response. But very little information is available on THC and DHC in relation to wound and application of antibiotics. Therefore the present work has been taken to investigate the effect of wounds on THC and DHC in adult insects of both sexes and finding are discussed.

Material and methods

The red cotton bug Dysdercus koenigii were collected as experimental insects from the cotton field and lady's finger cultivated field in the nearby villages. Insects were raised in glass jars in BOD incubator set at 28±1°C, 16 hr photoperiod and 75% RH. They were fed on water soaked cotton seeds. For the each experiment 0 hr of 20 adults were sorted out from cultured jars. Water narcotized insect were positioned in specially devised operating clamp under a binocular microscope. A transverse slit was made in the neck membrane with a razor-blade-scalpel and the fat body behind the brain was removed to make a severe wound. Controls were treated in similar manner but on their wound a drop of penicillin – streptomycin antibiotic (1:1 by weight) mixture dissolve in

insect Ringar was given that compensate the loss of protect wound from infection. The THC and DHC of both group insect were recorded at 24hr interval up to the day 8. Haemolymph or blood drop sample taken by cut the antennae of insect, for DHC at least 100 cells were chosen from random areas of stained blood smear and counted on a laboratory blood counter and their percentage of cells was calculated (Berger et al 2003, Mishra and Tiwari 2005).

Result

From the study of table 1, 2, 3, and 4 it is shown that in the insect group(wound without antibiotics) the differences in THC after 4^{th} day are almost at same trends in both groups. The THC in experimental group either with wound with antibiotics or wound without antibiotics shows lower value then to their respective control (without wound). The THC shows fluctuation on 4^{th} and 6^{th} day in controls. In experimental groups either wound with antibiotics or wound without antibiotics shows more population of PRs, GRs, OEs, VEs and less population of PRs, ADs to their respective controls. Fluctuation in DHC also observed mostly in PRs, PLs, and GRs during early days and in ADs and OEs during later days in all the group of insects.

Discussion

The lower THC in experimental insect is possibly either due to lack of haemolymph or due to restrictions of free circulation of haemocytes. Sometimes due to injury or shock circulating haemocytes goes in deep in circulation and aggregated in cellular spaces of body cavity or attach to tissues/connective tissues. In the present case both the reasons are evident because there is loss of haemolymph (without antibiotics) and restriction of haemocytes in circulation (wound with antibiotics). In insect (without antibiotics) have lower THC then their respective controls (wound with antibiotics) also support the earlier reasons. Further supported with higher THC in insects those not have wound. The fluctuation in THC on near 6^{th} day particularly in female possibly co-related with beginning of 1^{st} egg cycle and egg maturation because haemocytes are involved in transport of nutrients. This reason further strengthen with increase in PLs, GRs, ADs, and decrease in PRs. PLs somewhere involved in transport function while ADs in storage, GRs in tissue repair. The importance of PLs and GRs in defense is also clearly evident due to its higher population in experimental group. The fluctuation in GRs, ADs, PLs and OEs are somewhere due to inter conversion in one another. Possible role of VEs in defense cannot be ignored (Gupta 1979, Berger et al 2003, Greeny et al 2012, Pandey and Tiwari 2012).

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Results Table 1 Effect of wound (without application of antibiotic) on THC in adult

Adults	Age in days	Experimental THC/mm³ of haemolymph		Control THC/mm³ of haemolymph		P value
		Range	Mean ± SD	Range	Mean ± SD	
Female	1	3160-4440	3990.3 ± 189.5	3440-5110	4690.4±390.5	N.S
Male	1	2780-3610	3354.0 ± 210.5	2890-4040	3780.0 ± 280.7	N.S
Female	2	3210-4570	4010.0 ± 319.3	3660-5360	4880.0 ± 370.6	N.S
Male	2	2800-3690	3395.7 ± 280.4	2960-4370	3870.0 ± 295.4	N.S
Female	3	3280-4590	4032.7 ± 295.4	4050-5870	5510.0 ± 216.6	<0.01
Male	3	2810-3670	3410.0 ± 282.9	3240-4960	4470.5 ± 319.6	<0.01
Female	4	3310-4640	4110.6 ± 205.9	4220-6380	6024.0 ± 190.7	<0.01
Male	4	2880-3710	3490.0 ± 295.7	3400-5280	4740.0 ± 270.6	<0.01
Female	5	3640-5130	4690.7 ± 390.6	4260-6380	6110.0 ± 300	<0.01
Male	5	3400-5040	3990.0 ± 317.8	3510-5360	4810.1 ± 198.6	N.S
Female	6	4120-5400	4880.0 ± 359.3	4130-6290	6090.0 ± 305.6	<0.01
Male	6	3590-5180	4280.0 ± 217.8	3570-5390	4870.0 ± 201.4	N.S
Female	7	4600-6100	5390.6 ± 480.3	4070-5980	5880.0 ± 270.4	N.S
Male	7	4080-5200	4370.0 ± 410.3	3500-5360	4890.0 ± 210.6	N.S
Female	8	4360-6480	5580.6 ± 510.7	4100-6010	5900.0 ± 240.7	N.S
Male	8	4100-5290	4410 ± 392.8	3670-5490	4110.0 ± 207.6	N.S

Table 2 Effect of wound (Without Antibiotics) on DHC in adult

Adults	Age in days	Experimental % Haemocyte type ± SD						Control % Haemocyte type ± SD					
		PRs	PLs	GRs	ADs	OEs	VEs	PRs	PLs	GRs	ADs	OEs	VEs
Female	1	17.1 ± 1.35	37.2 ± 3.84	29.5 ± 2.78	16.6 ± 1.49	0	0	25.1 ± 2.38	34.4 ± 3.17	18.8 ± 1.57	19.3 ± 1.86	2.4 ± 0.03	0
Male	1	13.4 ± 1.23	32.1 ± 3.17	26.6 ± 2.86	24.1 ± 2.36	3.8 ± 0.13	0	19.6 ± 1.86	27.5 ± 2.58	28.4 ± 2.64	22.6 ± 2.45	1.9 ± 0.01	0
Female	2	14.6 ± 1.47	38.3 ± 3.86	29.4 ± 2.57	14.4 ± 1.57	2.1 ± 0.10	1.2 ± 0.10	22.1 ± 2.75	34.3 ± 3.79	18.7 ± 1.68	19.8 ± 1.75	4.1 ± 0.59	1.0 ± 0.10
Male	2	9.9 ± 9.08	38.1 ± 3.71	30.2 ± 3.21	15.8 ± 1.58	4.5 ± 0.53	1.5 ± 0.14	17.6 ± 1.68	29.3 ± 2.36	23.9 ± 2.76	25.5 ± 2.65	2.7 ± 0.31	1.0 ± 0.10
Female	3	14.9 ± 1.43	38.5 ± 3.71	30.0 ± 3.05	10.1 ± 1.05	4.9 ± 0.57	1.6 ± 0.17	18.6 ± 1.73	33.3 ± 3.86	16.2 ± 1.45	25.4 ± 2.78	5.3 ± 0.46	1.2 ± 0.10
Male	3	5.4 ± 0.58	48.2 ± 4.88	30.9 ± 3.17	4.4 ± 0.34	9.1 ± 0.91	2.0 ± 0.25	14.9 ± 1.25	29.1 ± 2.57	21.1 ± 2.19	30.5± 3.14	3.1 ± 0.16	1.3 ± 0.14
Female	4	10.1 ± 1.20	40.5 ± 4.56	31.6 ± 3.19	10.7 ± 1.13	5.0 ± 0.30	2.1 ± 2.8	17.2 ± 1.63	33.1 ± 3.19	16.1 ± 1.78	26.6 ± 2.33	5.5 ± 0.21	1.5 ± 0.12
Male	4	6.2 ± 0.88	47.1 ± 4.38	30.1 ± 3.56	5.7 ± 0.46	8.9 ± 0.89	2.0 ± 0.18	14.4 ± 1.28	28.2 ± 3.15	20.2 ± 1.89	32.1 ± 2.39	3.6 ± 0.23	1.5 ± 0.00
Female	5	8.3 ± 0.79	42.2 ± 4.21	34.2 ± 3.78	10.2 ± 1.05	5.1 ± 0.51	0	17.1 ± 1.39	31.7 ± 2.36	15.9 ± 1.73	29.4 ± 2.47	5.9 ± 0.57	0
Male	5	10.1 ± 1.06	39.9 ± 3.87	39.2 ± 3.89	4.5 ± 0.34	6.3 ± 0.38	0	14.1 ± 1.41	27.3 ± 2.47	20.1 ± 2.05	33.8 ± 3.26	4.7 ± 0.19	0
Female	6	10.5 ± 1.01	43.2 ± 4.25	35.1 ± 3.49	9.1 ± 0.98	2.1 ± 0.02	0	16.9 ± 1.39	30.8 ± 3.15	15.7 ± 1.34	29.7 ± 2.56	6.9 ± 0.38	0
Male	6	13.6 ± 1.23	36.5 ± 3.63	42.4 ± 4.73	2.7 ± 0.17	4.8 ± 0.38	0	13.9 ± 1.76	27.1 ± 2.68	19.7 ± 1.86	33.4 ± 3.76	5.9 ± 0.45	0

Female	7	10.9 ± 1.03	44.5 ± 4.86	32.2 ± 4.26	11.3 ± 1.03	1.1 ± 0.03	0	16.6 ± 1.32	28.6 ± 2.57	15.4 ± 1.43	32.7 ± 3.68	6.7 ± 0.63	0
Male	7	11.4 ± 1.34	38.7 ± 3.75	38.9 ± 3.74	5.4 ± 0.29	5.6 ± 0.47	0	10.4 ± 1.03	25.8 ± 2.38	18.7 ± 1.59	37.5± 3.81	7.6 ± 0.81	0
Female	8	12.8 ± 1.27	43.4 ± 4.57	29.5 ± 2.57	14.3 ± 1.23	0	0	11.5 ± 1.14	26.3 ± 2.76	14.3 ± 1.34	37.2 ± 3.86	10.7 ± 0.78	0
Male	8	10.7 ± 1.01	36.4 ± 3.46	38.0 ± 3.25	8.6 ± 0.89	6.3 ± 0.54	0	10.2 ± 1.03	25.3 ± 2.46	16.6 ± 1.57	39.6± 3.98	8.3 ± 0.69	0

Table 3 Effect of wound (with application of antibiotic) on THC in adult

Adults	Age in days	Experimental THC/mm³ of haemolymph		Control THC/mm³ of haemolymph		P Value
		Range	Mean ± SD	Range	Mean ± SD	
Female	1	3760-5090	4640.0 ± 395.7	4020-5570	5180.0 ± 210.6	N.S
Male	1	2890-4100	3760.0 ± 278.6	3000-4360	4108.3 ± 310.8	<0.01
Female	2	3660-5360	4790.0 ± 372.7	4620-6160	5790.8 ± 290.8	N.S
Male	2	2900-4340	3870.0 ± 292.8	3240-4760	4240.0 ± 284.3	N.S
Female	3	4090-5870	5530.0 ± 210.6	5180-6330	6040.0 ± 380.7	N.S
Male	3	3200-4950	4430.6 ± 330.7	3400-4800	4690.0 ± 278.4	N.S
Female	4	4200-6400	6040 ± 201.4	4710-6420	6210.0 ± 405.8	N.S
Male	4	3420-5280	4705.0 ± 276.8	3500-4940	4780.0 ± 360.4	N.S
Female	5	4300-6290	6110.0 ± 296.4	4500-6600	6480.0 ± 490.7	N.S
Male	5	3500-5350	4830.0 ± 203.7	3580-5460	5040.0 ± 298.7	N.S
Female	6	4100-6300	6070 ± 317.4	4670-6890	6780.0 ± 307.8	N.S
Male	6	3500-5400	4840.0 ± 208.2	3570-5500	5110.0 ± 302.8	<0.01
Female	7	4100-6110	5840.2 ± 278.6	4300-6700	6740.0 ± 201.4	N.S
Male	7	3500-5280	4880.0 ± 222.7	3540-5530	5010.0 ± 308.4	N.S
Female	8	4120-6010	5890.0 ± 252.9	4200-6400	5960.3 ± 222.9	N.S
Male	8	3660-5480	4110.0 ± 211.4	3590-5530	4690.0 ± 390.7	N.S

Table 4 Effect of wound (with antibiotic) on DHC in adult

Adults	Age in days	Experimental % Haemocyte type ± SD						Control % Haemocyte type ± SD					
		PRs	PLs	GRs	ADs	OEs	VEs	PRs	PLs	GRs	ADs	OEs	VEs
Female	1	15.6 ± 1.35	37.5 ± 3.84	29.3 ± 2.78	17.6 ± 1.49	0	0	26.2 ± 2.38	33.7 ± 3.17	18.8 ± 1.57	19.1 ± 1.86	2.2 ± 0.03	0
Male	1	12.9 ± 1.23	31.9 ± 3.17	26.8 ± 2.86	24.5 ± 2.36	3.9 ± 0.13	0	19.8 ± 1.86	26.9 ± 2.58	28.1 ± 2.64	23.3 ± 2.45	1.9 ± 0.01	0

Female	2	14.7 ± 1.47	38.8 ± 3.86	28.9 ± 2.57	14.2 ± 1.57	2.2 ± 0.10	1.2 ± 0.10	22.3 ± 2.75	34.4 ± 3.79	18.7 ± 1.68	19.8 ± 0.75	4.8 ± 0.59	0
Male	2	9.8 ± 9.08	37.9 ± 3.71	30.4 ± 3.21	15.8 ± 1.58	4.9 ± 0.53	1.2 ± 0.20	17.1 ± 1.68	29.6 ± 2.36	24.4 ± 2.76	26.1 ± 2.65	2.8 ± 0.31	0
Female	3	14.9 ± 1.43	38.7 ± 3.71	30.0 ± 3.05	10.2 ± 1.05	5.2 ± 0.57	1.0 ± 0.10	19.1 ± 1.73	33.4 ± 3.86	16.2 ± 1.45	26.2 ± 2.78	5.1 ± 0.46	0
Male	3	6.2 ± 0.58	48.7 ± 4.98	31.2 ± 3.17	4.5 ± 0.39	9.4 ± 0.91	0	15.1 ± 1.25	28.9 ± 2.57	21.5 ± 2.9	31.1 ± 3.14	3.3 ± 0.16	0
Female	4	10.3 ± 1.30	41.6 ± 4.56	32.0 ± 3.19	10.8 ± 1.13	5.3 ± 0.39	0	17.9 ± 1.63	33.2 ± 3.86	16.6 ± 1.78	27.0 ± 2.33	5.3 ± 0.21	0
Male	4	7.0 ± 0.88	47.6 ± 4.38	30.3 ± 3.56	5.8 ± 0.46	9.3 ± 0.89	0	15.1 ± 1.38	28.5 ± 3.15	20.3 ± 1.89	32.3 ± 2.39	3.8 ± 0.23	0
Female	5	8.5 ± 0.79	41.9 ± 4.21	34.4 ± 3.78	10.2 ± 1.05	5.0 ± 0.51	0	17.4 ± 1.39	31.5 ± 2.36	16.1 ± 1.73	29.2 ± 2.49	5.8 ± 0.57	0
Male	5	10.1 ± 1.06	39.8 ± 3.87	39.4 ± 3.89	4.3 ± 0.34	4.4 ± 0.38	0	14.9 ± 1.41	27.3 ± 2.47	20.1 ± 2.05	33.5 ± 3.26	4.2 ± 0.19	0
Female	6	10.5 ± 1.01	42.9 ± 4.25	35.2 ± 3.49	9.3 ± 0.98	2.1 ± 0.02	0	16.9 ± 1.36	30.8 ± 3.15	15.9 ± 1.34	29.5 ± 2.56	6.9 ± 0.38	0
Male	6	14.1 ± 1.23	37.1 ± 3.63	41.7 ± 4.73	2.6 ± 0.17	4.5 ± 0.38	0	14.3 ± 1.26	27.1 ± 2.68	19.8 ± 1.86	32.9 ± 3.76	5.9 ± 0.45	0
Female	7	10.8 ± 1.03	44.6 ± 4.86	32.3 ± 4.26	11.2 ± 1.03	1.1 ± 0.38	0	16.3 ± 1.32	28.6 ± 2.57	15.2 ± 1.43	33.1 ± 3.68	6.8 ± o.63	0
Male	7	11.3 ± 1.34	38.4 ± 3.75	39.2 ± 3.74	5.6 ± 0.29	5.5 ± 0.47	0	10.4 ± 1.03	25.9 ± 2.38	18.6 ± 1.59	37.6 ± 3.31	7.5 ± 0.81	0
Female	8	13.8 ± 1.29	42.2 ± 4.57	29.6 ± 2.57	14.4 ± 1.23	0	0	12.1 ± 1.14	25.8 ± 2.76	14.1 ± 1.34	37.1 ± 3.86	10.9 ± 0.78	0
Male	8	10.6 ± 1.01	36.3 ± 3.46	38.2 ± 3.25	8.6 ± 0.89	6.3 ± 0.54	0	10.1 ± 1.03	25.4 ± 2.46	16.3 ± 1.57	39.8 ± 3.9	8.4 ± 0.68	0