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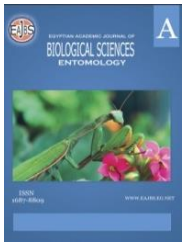

Effect of Different Host Plants on The Different Haemocyte Counts and Haemocyte Viability of Larvae of *Spodoptera littoralis* and *Agrotis ipsilon.*

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ABSTRACT

The present study aimed to investigate the effect of different six host plants namely, Cabbage, alfalfa, Jaw's mallow, lettuce, turnip, and castor leaves on the total haemocyte count (THC), percentage of haemocyte viability, and the percentage of each type of haemocyte (differential haemocyte count, DHC) in *Spodoptera littoralis* and *Agrotis ipsilon* fourth instar larvae. There are five types of haemocytes in *A. ipsilon* and *S. littoralis* larvae; Prohaemocyte, Plasmatocyte, Granulocyte, Spherocytes, and Oenocyte. Results recorded the highest value 13.3±0.68 of Prohaemocyte at lettuce and the lowest value 9.3 for Cabbage, alfalfa and turnip. On the other hand, Prohaemocyte recorded the highest value 12.6 ± 0.9 in the case of castor feeding and 8.7 ± 0.3 for turnip for *S. littoralis*. Plasmatocyte recorded 45.3 ± 2.9 cells/mm^{3}for *A. ipsilon* feed on turnip and 47.3 ± 1.8 cells/mm^{3}for *S. littoralis* feed on lettuce as the highest value while Granulocyte recorded 33 ± 4.1 and 35 ± 0.7 for *A. ipsilon* feed on Jaw's mallow, respectively. Spherocytes recoded 15.3 ± 0.9 and 12.3 ± 0.9 for *A. ipsilon* and *S. littoralis* feed on alfalfa. Finally, Oenocyte recorded 7.7 ± 1.5 and 7.3 ± 1.4 for *A. ipsilon* and *S. littoralis* feed on castor . Total haemocytic count recorded 29 ± 0.3 and 36 ± 0.75 cells/mm^3 as the highest value when both *A. ipsilon* and *S. littoralis* feed on Alfalfa. Also, the haemocyte viability % recorded the highest value 91.5 ± 2.6 and 96.2 ± 0.8 % for *A. ipsilon* and *S. littoralis* feed on feed on Alfalfa. The result recorded increasing DHC level after feeding of the fourth larval instar of *S. littoralis* and *A. ipsilon* on different plant hosts such an increase in DHC gives an impression that blood cells may share in detoxifying. The most obvious activity of haemocyte is phagocytosis. In this context, the most active phagocytes are the Plasmatocyte, Plasmatocyte which was implicated in the encapsulation of necrotic tissues.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), and the black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) are key pests in most of the countries causes extensive economic losses in many cultivated crops. The normal haemocyte of caterpillars was thorough- ally investigated and classified into five main groups (Prohaemocyte, spherocytes, Plasmatocyte, Granulocytes, and Oenocytes) by several authors (Cameron, 1934; Steinhaus, 1949; Wigglesworth, 1959; Nittona, 1960; Habib, 1977; Kim *et al.*, 1990;

Strand and Noda, 1991; Kurihara *et al.*, 1992; El – Kattan, 1995; Amin, 1998, EL-Sheikh 2002 and Abdel – aal 2003). Haemolymph is a fluid, analogous to the blood in vertebrates that circulates in the interior of the arthropod body remaining in direct contact with the animal's tissues. It is composed of fluid plasma in which haemolymph cells called haemocyte are suspended. Insects have a haemocoele, in which the only tissue fluid, the blood, circulates. The blood consists of haematocytes and plasma. Most of the hepatocytes are phagocytic leucocytes. Insects possess an effective innate immune system against foreign organisms. Innate immunity of insects is divided into two major reaction types: humeral and cellular reactions humeral reactions involve cellular proteins in the haemolymph such as phenoloxidase, antimicrobial proteins (AMPs), lysosomes, and lectins, whereas haemocyte mediates cellular reactions such as phagocytosis, encapsulation, and nodule formation Tanka and Amakawa (2011).

MATERIALS AND METHODS

Experiment Insects:

The colony of the cotton leafworm, *S. littoralis,* and black cutworm, *A. ipsilon* were obtained from Plant Protection Research Institute, Ministry of Agriculture Dokki, Giza. Without any insecticidal contamination. The larvae were fed on castor oil leaves*, Ricinus communis*, L. maintained at 25 ± 2ºC and 70 ± 5 % RH. The rearing technique was the same adopted by El**-** Defrawi *et al.,* 1964).

Host Plants Used:

The stock culture of susceptible Egyptian cotton leafworm, *S. littoralis* (Boisd.) and the black cutworm, *A. ipsilon* (Hufn) were reared on six different host plants, jaw's mallow, *Corchorus ditorius* (Family: Tillaceae), cabbage, *Brassica oleracea* (Family: Brassicaceae), turnip, *Brassica campostris var. rapa* (Family: Brassicaceae), lettuce, *Lactuca sativa* (Family: Asteraceae), alfalfa, *Medicago sativa* (Family: Fabaceae) and castor bean leaves, *Ricinus communis* (Family: Euphorbiaceae), Choice of host plants was based on the fact that some of the hosts are heavily attacked in fields while the other is preferred for oviposition (Moufied *et al.,* 1960). Haemolymph samples analysis were carried out in physiological laboratory analysis of Plant Protection Research Institute Ministry of Agriculture Dokki, Giza

Different Types of Haemocytes:

One drop of fresh haemolymph of 4th instar larvae of *S. littoralis* & *A. ipsilon* which fed on six different host plants (cabbage, pepper, lettuce, turnip, castor leaves, and jaw's mallow was smeared on a clean glass slide, air-dried and then fixed for 2 min with ethanol. Blood films were stained with Giemsa stain. Freshly prepared by mixing stock Giemsa with distilled water (1:10) (v/v) for 15 min. Slides were dipped for about 30 sec in tap water. Blood smears were air-dried for 24 h, mounted in Canada balsam, and then examined under a light microscope (100 x magnifications). Different types of haemocytes were classified according to methods of Jones, (1964).

Total Haemocytes Count and Viability:

Haemolymph from the pooled sample (10 fourth instar larvae) was taken up directly by Thoma white- blood cell diluting pipette to 0.1 marks. The diluting solution was taken up to the 11th mark on the pipette. The mixture was hand-shaken for three min and then dispensed to both chambers of Neubauer haemocytometer (DHC-No1). After one min, the total number of blood cells in 64 squares of the four corners was recognized as viable and dead cells. Dead haemocytes were stained with trypan blue, whereas viable cells were not (Horohov and Dunn, 1982). Cells within the lines and at the left and bottom boundary lines of the four corner squares were counted. The total number of cells was multiplied by a factor

of 250 to give the number of cells/mm3 of haemolymph. This procedure was replicated 10 times for each treatment. The number of circulating haemocytes per cubic millimeter was calculated by the formula suggested by Jones (1962). The percentage of viability was calculated according to Horohov and Dunn (1982).

RESULTS AND DISCUSSION

Differential Haemocytes Counts:

The differential haemocyte counts (DHC%) of the 4th instar larvae of A. ipsilon feed on Cabbage, alfalfa, Jaw's mallow, lettuce, turnip, and castor leaves are shown in Tables (1, 2,3 and 4) and illustrated in Figs. (1, 2, 3 and 4) recorded the highest value 13.3 ± 0.68 of Prohaemocyte at lettuce and the lowest value 9.3 for Cabbage, alfalfa, and turnip, on the other hand, Prohaemocyte recorded the highest value 12.6 ± 0.9 in the case of castor feeding and 8.7 ± 0.3 for turnip for *S. littoralis.* Plasmatocyte recorded 45.3 ± 2.9 cells/$mm^3$for *A. ipsilon* feed on turnip and 47.3 ± 1.8 cells /mm^3 for S. littoralis feed on lettuce as the highest value while Granulocyte recorded 33 ± 4.1 and 35 ± 0.7 for A. ipsilon feed on Jaw's mallow, respectively. Spherocytes recoded 15.3 ± 0.9 and 12.3 ± 0.9 for *A. ipsilon* and *S. littoralis* feed on alfalfa. Finally, Coenocyte recorded 7.7 ± 1.5 and 7.3 ± 1.4 for *A. ipsilon* and *S. littoralis* feed on castor leaves, respectively. Total haemocytic count recorded 29 ± 0.3 and 36 ± 0.75 cells/mm^3 as the highest value when both *A. ipsilon* and *S. littoralis* feed on Alfalfa.

Haemocyte Viability Percentage:

The Haemocyte viability % recorded the highest value of 91.5 ± 2.6 and 96.2 ± 0.8 % for A. ipsilon and S. littoralis feed on feed on alfalfa, respectively. The result recorded increasing DHC level after feeding of the second larval instar of S. littoralis and *Agrotis ipsilon* on different plant hosts such an increase in THC gives an impression that blood cells may share in detoxifying. Patton, (1961) implicated that Haemocyte may function in detoxification in the present study the cause of such stimulus might be as the result of feeding on a different host plant.

The most obvious activity of haemocyte is phagocytosis. In this context, the most active phagocytes are Plasmatocyte Jones, (1962). Plasmatocyte was implicated in the encapsulation of necrotic tissues Essawy, (1990). Abdel-Aal, (2003) recorded 8.6 ± 0.01, 27.9 ± 0.02, 33.40 ±0.03, 23.3 ± 0.03and 6.8 ± 0.01 for Prohaemocyte, Plasmatocyte, Granulocyte, Spherocytes, and Coenocytes *S. littoralis* feed on castor oil leaves. Also, Elshikh, (2002) recorded11.7 ± 0.4, 61.4 ± 0.67, 8.2 ± 0.39, 14.1 ± 0.3, and 4.6 ± 0.31 for Prohaemocyte, Plasmatocyte, Granulocyte, Spherocytes and Oenocytes in *A. Ipsilon* feed on castor leaves.

Table 1: Effect of different host plants on the different haemocyte counts of 4th instar larvae of *A. ipsilon.*

Host plants	Prohaemocyte	Plasmatocyte	Granulocyte	Spherocyte	Oenocyte
	Mean± S. E				
Cabbage	9.3 ± 0.89	42.6 ± 1.5	29.3 ± 89	14.3 ± 1.5	4.3 ± 0.9
Alfalfa	9.3 ± 0.34	38.7 ± 1.2	32.7 ± 0.67	15.3 ± 0.9	7.3 ± 2.6
Jew's mallow	11.66 ± 0.33	38.68 ± 0.9	33 ± 4.1	12.3 ± 2.7	4.3 ± 2
Lettuce	13,3 ± 0.68	41 ± 3.05	32.3 ± 3.3	9.7 ± 1.8	3.7 ± 0.7
Turnip	9.3 ± 1.4	45.3 ± 2.9	29 ± 1.6	11.7 ± 1.9	4.7 ± 1.2
Castor leaves	11.6 ± 2.6	41.0 ± 3.9	29 ± 4.4	11 ± 2.4	7.7 ± 1.5

Table 2: Effect of different host plants on the different haemocyte counts of 4th instar larvae of *S. littoralis*

Host plants	Prohaemocyte	Plasmatocyte	Granulocyte	Spherocyte	Oenocyte
	Mean± S.E				
Cabbage	9.0 ± 1.2	42.6 ± 0.7	34.3 ± 1.2	9.3 ± 0.3	4.7 ± 0.7
Alfalfa	8.7 ± 0.7	38.7 ± 1.9	32.7 ± 1.4	12.3 ± 0.9	7.7 ± 1.4
Jew's mallow	10.3 ± 1.8	41.3 ± 2.0	35.6 ± 0.7	8.7 ± 0.9	4 .0 ± 0.0
Lettuce	9.3 ± 0.9	47.3 ± 1.8	29.3 ± 4.1	11.0 ± 2.0	3.6 ±0.3
Turnip	8.7 ± 0.3	41.3 ± 1.4	32.7 ± 1.4	10.3 ± 1.2	7.0 ± 0.6
Castor leaves	12.6 ± 0.9	39.0 ± 3.2	30.6 ± 1.2	10.3 ± 1.9	7.3 ± 1.4

Table 3: Effect of different host plants on the total haemocyte counts of 4th instar larvae of *A. ipsilon* and *S. littoralis*.

Host plants	Total haemocyte counts(cells/mm3) X103*	
	Mean± S.E	
	A. ipsilon	*S. littoralis*
Cabbage	23.8 ± 1.3	30.7 ± 2.0
Alfalfa	29.3 ± 0.3	36.75 ± 2.3
Jew's mallow	22.0 ± 0.6	29.0 ± 2.0
Lettuce	23.2 ± 0.6	29.75 ± 2.0
Turnip	24.6 ± 0.6	30.75 ± 2.0
Castor leaves	23.9 ± 0.7	32.75± 2.0

Table 4: Effect of different host plants on the haemocyte viability % of 4th instar larvae of *A. ipsilon* and *S. littoralis*.

Host plants	Haemocyte viability %	
	Mean± S. E	
	A. ipsilon	*S. littoralis*
Cabbage	92.4 ± 0.45	84.5 ± 2.3
Alfalfa	96.2 ± 0.8	91.5 ± 2.6
Jew's mallow	87.4 ± 1.7	81.38 ± 2.3
Lettuce	88.3 ± 1.7	79.3 ± 2.2
Turnip	77.7 ± 0.9	87.7 ± 2.4
Castor leaves	87.9 ± 1.9	85.3 ± 2.4

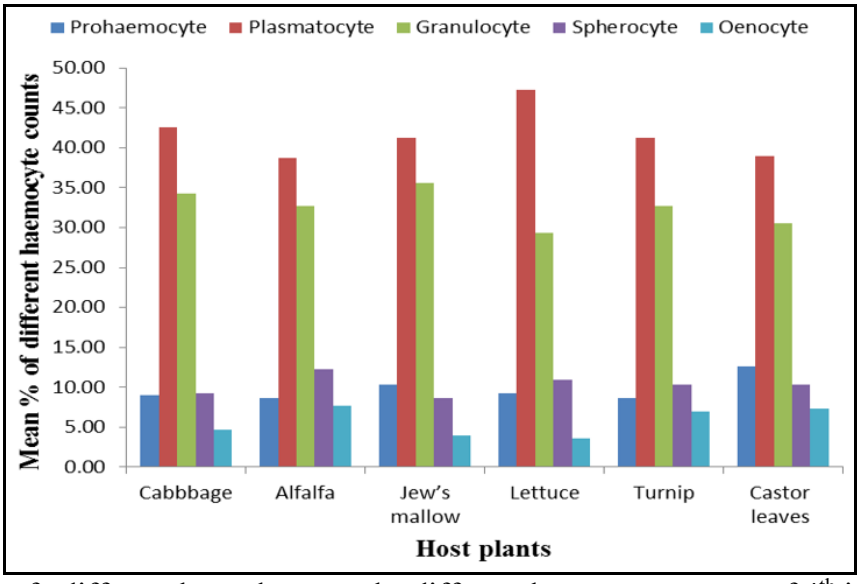

Fig. 1: Effect of different host plants on the different haemocyte counts of 4th instar larvae of *S. littoralis.*

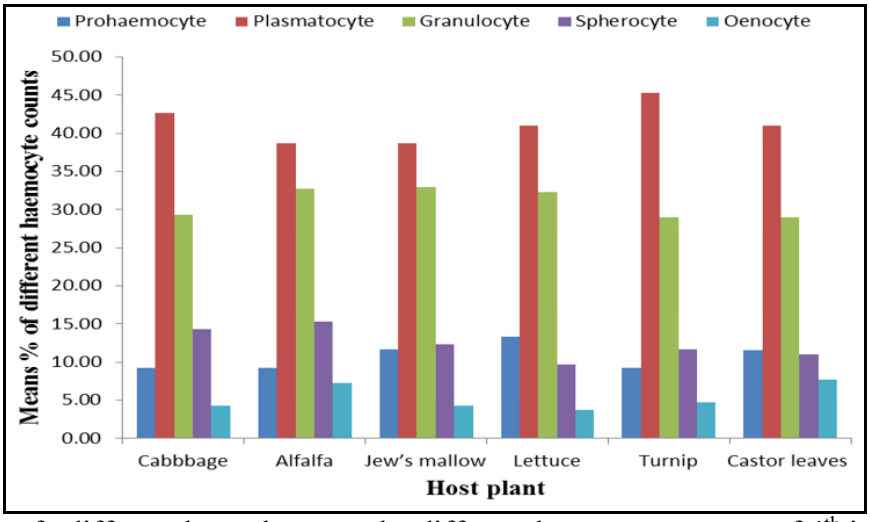

Fig. 2: Effect of different host plants on the different haemocyte counts of 4th instar larvae of *A. ipsilon*

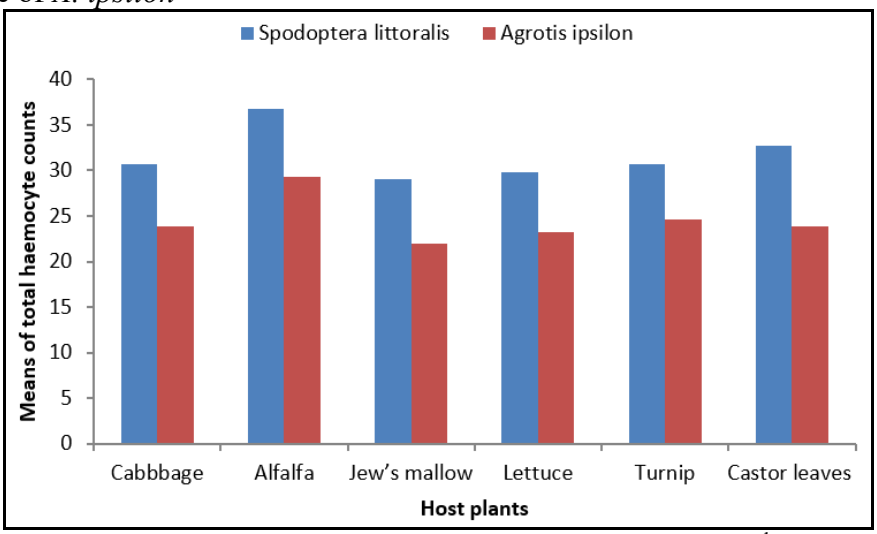

Fig. 3: Effect of different host plants on the total haemocyte counts of 4th instar larvae of *S. littoralis* and *A. ipsilon*.

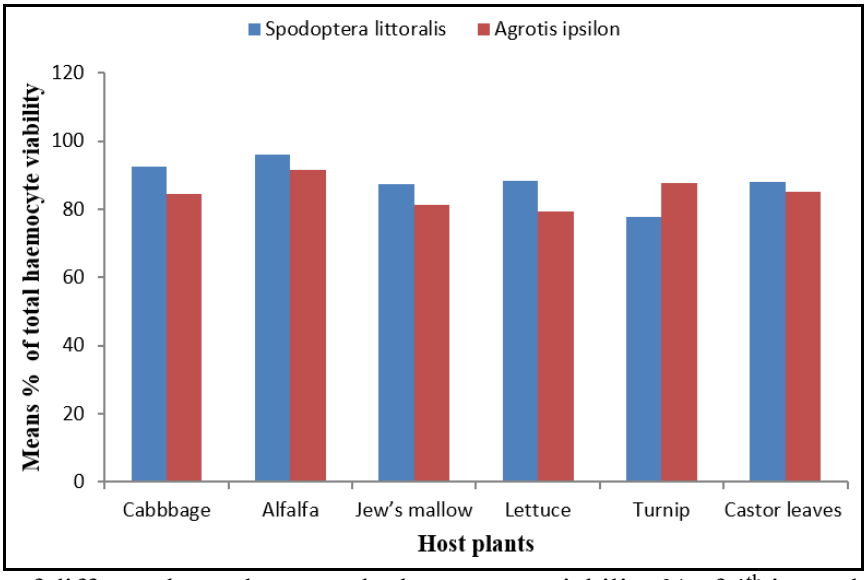

Fig. 4: Effect of different host plants on the haemocyte viability % of 4th instar larvae of *S. littoralis* and *A. ipsilon*.

REFERENCES

Abdel-Aal, A .E. (2003): Effect of some insect growth regulators on some biological, biochemical and histopathological aspects of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Ph. D., Thesis Faculty of Science, Cairo University.* 170 pp.

Amin, T. (1998): Biochemical and physiological studies on the cotton leafworm, *Spodoptera littoralis* (Boisd.). *Ph. D., Thesis, Faculty of Science, Cairo University, Egypt,* 198 pp.

Cameron, G. R. (1934): Inflammations in the caterpillars of Lepidoptera. *Journal of Pathology and Bacteriology,* 38: 441-446.

El-Kattan, N. A. I. (1995): Physiopathological studies on the Indian meal moth, *Plodia interpunctella* HB. (Pyralidae: Lepidoptera). Infected with microbial entomophathogens. *Ph.D., Thesis, Fac. Sci., Ain Shams Univ., Egypt*. 217 pp.

El- Defrawi, M.E., A. Tappozada, N. Mansour and M. Zeid (1964): Toxicological studies on the Egyptian cotton leafworm *Prodenia litura* L. Susceptibility of different larval instars of *Prodenia* to insecticides. *Journal of Economical Entomology*, 57:591- 593

El-sheikh T. A. (2002): Effects of application of selected insect growth regulators and plant extracts on some physiological aspects of the black cutworm, *Agrotis ipsilon* (Huff.). *Ph. D., Thesis Faculty of Science, Ain Shams University*. 219 pp.

Essawy, M. M. (1990): Changes in the differential haemocyte counts of the last larval instar of *Spodoptera littoralis* (Boisd.) during wound healing. *Alexandria Science Exchange Journal,* 2 (4): 151-169.

Habib, M. E. M. (1977): Haemolymph and haemocyte of the full grown larvae of *Alabana argillacea* (Hubner, 1818) (Lepidoptera: Noctuidae). *Annals Society of Entomology,* 6: 25-31.

Horohov D.W. and Dunn P.E. (1982): Changes in the circulating haemocyte population on *Manduca sexta* larvae following injection of bacteria. *Journal of Invertebrate Pathology,* 40, 327- 339.

Jones, J. C. (1962): Current concepts concerning insect haemocyte. *American. Zoologist*, 2: 209-246.

Jones J.C. (1964): Differential haemocyte counts from unfixed last stage *Galleria mellonella*.

American Zoologis, 4, 337- 346.
Kim, C. S.; Yoon, I. B. and Kim, W. K. (1990): Ultrastructure of the haemocyte in *Lymantria dispar* L. *Korean Journal of Entomology*, 20: 223-230.
Kurihara, Y. T.; Shimazu, T. and Wago, H. (1992): Classification of haemocyte in the common cutworm, *Spodoptera littura* (F.) (Lepidoptera: Noctuidae). I. Phase microscopic study. *Applied Entomology Zoology*, 27: 225-235.
Moufied, A. M.; Zaher, M. A. and Kotby, Fawkia (1960): Abundance of cotton leafworm in relation to host plants. *Bulletin. Society of Entomology. Egypt, XLIV pp.* 240-251.
Nittona, Y. (1960): Studies on the blood cells in the silk worm, *Bombyx mori*. *Bulletin. Sericultural Expperiment Station Japan,* 15: 171-226. Patton, R. L. (1961): The detoxification functions of insect haemocyte. *Annals. Entomology. Society of American,* 54: 696-698.
Steinhaus, E. A. (1949): Principles of insect pathology. Mc Grew Hill, New York, pp. 757.
Strand, M. R. and Noda, T. (1991): Alternations in the haemocyte of *Pseudoplusia includens* after parasitism by *Microplitis demolitor*. *Journal of Insect Physiology*, 37: 839-850.
Tanaka H and Yamakawa M (2011): Regulation of the innate immune responses in the silkworm, *Bombyx mori*, ISJ8:59- 69.
Wigglesworth, V. B. (1959). Insect blood cells. *Annals Review Entomology,* 4: 1-6.

ARABIC SUMMARY

تأثير العوائل النباتية المختلفة على العدد النوعى ونسبة بقاء كريات الدم فى يرقات دودة ورق القطن والدودة القارضة

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تم فحص خلايا الدم الطبيعية من اليرقات بدقة وتصنيفها إلى خمس مجموعات رئيسية وهى (الخلايا الأولية ، والخلايا الكروية ، والخلايا البلازمية ، والخلايا المحببة ، والخلايا العينية) ، وقد سجل تعداد خلايا الدم النوعيه (DHC%)من يرقات الطور السادس ليرقات الدوده القارضه المتغذية على الكرنب ، البرسيم ، الملوخية ، الخس ، اللفت ، الخروع حيث كانت أعلى قيمة 13.3 ± 0.68 من الخلايا الأولية في الخس وكانت أقل قيمة 9.3 للكرنب ، البرسيم ، سجلت الخلايا الأولية أعلى قيمة 12.6 ± 0.9 في حالة التغذية على الخروع و 8.7 ± 0.3 ، بالنسبة لدودة ورق القطن سجلت Plasmatocyte 45.3 ± 2.9 خلية / مم 3 عند التغذية على الخس كأعلى قيمة بينما سجلت Granulocyte 33 4.1 ±و 35 ± 0.7 لتغذية الدودة القارضة عند التغذية على الملوخية على التوالي.
الخلايا الكروية المعاد ترميزها 15.3 ± 0.9 و 12.3 ± 0.9 لكل من دودة ورق القطن والدودة القارضة على البرسيم، وسجلت coenocyte 7.7 ± 1.5 و 7.3 ± 1.4 لتغذية يرقات الحشرتين على الخروع. سجل إجمالي عدد خلايا الدم 29 ± 0.3 و 36 ± 0.75 خلية / مم كأعلى قيمة عند تتغذية يرقات كل من دودة ورق القطن و الدودة القارضة على البرسيم. كما سجلت نسبة بقاء الخلايا الدموية أعلى قيمة 91.5 ± 2.6 و 96.2 ± 0.8٪ عند تغذية يرقات كل من دودة ورق القطن و الدودة القارضة على البرسيم. النشاط الأكثر وضوحا للخلايا الدموية هو البلعمة، في هذا السياق فإن البلعمات الأكثر نشاطًا هي الخلايا البلازمية.