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Influence of Tylosin Drug on Blood Cells of Honeybee Worker Larvae

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ABSTRACT

The primary objective of this work was to study the effect of tylosin on the honeybee larvae blood cells. Honeybee colonies were treated with a solution of tylosin. Larvae blood samples were collected and pulled directly onto glass and examine with 1250x microscope. In mostly the blood cells count were decreased except in prohaemocytes and oenocytes, in both treatments factors (time and larvae instar). The third day after treated with tylosin showed the highest amount of Oenocytes (12.67±4.04) in the third instar. While the lowest amount ((5.67±3.21) was recorded in the control. The Oenocytes cells in the second larvae Instar were increased and reached 10.67±2.31 while the control amount was 5.67±3.21. The results showed that, tylosin has strong effect on the blood cells count. And may be decreased the honeybee immunity.

Keywords: tylosin, blood cells, Plasmatocytes, prohaemocytes, Coagulocytes, Spherule, Oenocytes.

INTRODUCTION

Tylosin is an antibacterial widely used drug in beekeeping, derived from Bacteria. Recently it was used to treated honeybee foul brood diseases. As the immunity of the insect generally dependent on some blood cells. Tylosin is the naturally macrolide veterinary antibiotic produced by the actinomycete *Streptomyces fradiae* as a mixture of tylosin A, tylosin B, tylosin C and tylosin D, it is active against most gram-positive bacteria, mycoplasma and certain gram-negative bacteria. Tylosin was the most used antibiotic at 31% of swine production facilities (Bush *et al*., 2001), and it was detected in 14% of 139 streams in United Stated of America (Kolpin *et al*., 2002). It is a widely antibiotic used for therapeutics in honeybee. It is used at sub-therapeutic levels to prevent honeybee diseases. After administration, up to 90% of drugs can be excreted and adsorb to bee hive part, different honeybee body feces and honeybee products as metabolites or in the parent form. Through the application of treatment disease antibiotics enter into every part of honeybee colony. And may effected on the honeybee immunity by stimulated its blood cells. The tylosin intensive used led to microorganism resistance.

Through application of manure with excreted tylosin residues from livestock on croplands, tylosin enters the water system as a pollutant in the environment. Since tylosin and its related compounds have been shown to have biological activity (Teeter *et al*., 2003), the fate and impact of tylosin in the environment has been recently getting more attention (Teeter *et al*., 2003, Loke *et al*., 2000, Ingerslev *et al*., 2006, Rabolle *et al*., 2000, De Liguoro *et al*., 2003, and Hu D *et al*., 2007). This study aimed to study Tylosin drug effect on honey bee worker larvae blood cells count.

MATERIALS AND METHODS

1. Three experimental local honeybee colonies equal in strength were used.
2. Sucrose solution 50% (1 sugar- 1 water)
3. Tylosin (tylosin tartrate) Manufactured by South Egypt Drug Industries company (SEDICO)
4. Glass and cover slides
5. Wright's blood stains and Buffer solution
6. 1250 x microscope
7. Seder oil

Procedure:

1. Blood stains: 25 ml methyl alcohol add to 100 ml ethyl alcohol add to g powder Wright's stain then shacked and filtering with filter paper.
2. Buffer solution: 3.315 monobasic potassium phosphate add to 1.28 dibasic sodium phosphate add to 500 ml distilled water.
3. The honeybee colonies were treated with a solution of 0.5 g of tylosin tartrate in 100 mL aqueous sucrose solution (50% w/v) by pouring into. Blood samples were collected among 3 days after treatment, every day three hive as replicates of the larvae age 2-3-4-5 days then, it pulled the larvae blood samples directly onto glass slides.
4. Blood samples: Puncturing the larvae abdomen with a fine scissor. The haemolymph was allowed to fall on a glass slide, and then a smear was made (Shapiro 1968). The smears were allowed to dry and then stained using wright's blood stains by placed into the jar of Wright's stain for 2-5 minutes and then transferred directly to the jar containing the buffer for 2-5 minutes. The slide is then rinsed with buffer or distilled water and air dried according to (Salinger 1963 and Arnold *et al*., 1976). The smears were examined under oil immersion at 1250x and maximum of 100 haemocytes/slide were differentiated using the classification of (Jones 1962 and Akai *et al*., 1973).

RESULTS AND DISCUSSION

It was clearly too now that, every blood cell type has a clear job for example, oenocytes blood cells are conceder as one of the main factor response abut animal immunity.

Results in table (1) showed the differential blood cells count of honeybee larvae second, third, fourth and fifth instars treated with 0.5 g of tylosin during three days.

Prohaemocytes At the first treated day showed that, the second and fifth larvae instar showed the highest amount (25±2.08 and 25±4.62 respectively). As the lowest amount of prohaemocytes (20±5) was recorded in the third larvae instar. At the second day, the fourth instar showed the highest amount of prohaemocytes (25±6.25). The lowest amount of prohaemocytes (20.67±3.21) was recorded in the second larvae instar. The third applied day with tylosin showed the highest amount of prohaemocytes (26±1.7) in the

second larvae instar. While the lowest amount (18.67±3.05) was recorded in the fourth larvae instar.

Plasmatocytes At the first treated day showed that, the control has the highest amount (26±3). As the lowest amount of Plasmatocytes (14±2.64) was recorded in the fourth larvae instar. At the second day, the control noted the highest amount of prohaemocytes (26±3). The lowest amount of Plasmatocytes (16.3±3.79) was recorded in the second larvae instar. The third applied day with tylosin showed the highest amount of Plasmatocytes (26.67±3.5) in the second larvae instar. While the lowest amount (11.67±6.51) was recorded in the fifth larvae instar.

Coagulocytes with granules at the first treated day with tylosin showed that, the control has the highest amount (11.67±3.06). As the lowest amount of Coagulocytes With granules (8.67±3.5) was recorded in the second larvae instar. After 48 hours, the fifth larvae instar noted the highest amount of Coagulocytes with granules (24±2.65). The lowest Coagulocytes With granules amount (10±0.00) was recorded in the second larvae instar. The third day after treated with tylosin showed the highest amount of Coagulocytes With granules (11.67±3.06) in the control. While the lowest amount (6.67±1.5) was recorded in the second larvae instar.

Coagulocytes Without granules at the first treated day with tylosin showed that, the control has the highest amount (25.33±9.07). As the lowest amount of Coagulocytes Without granules (4±1) was recorded in the fourth larvae instar. After 48 hours, the control has the highest amount (25.33±9.07). The lowest Coagulocytes Without granules amount (4.67±2.08) was recorded in the third larvae instar. The third day after treated with tylosin showed the highest amount of Coagulocytes Without granules (25.33±9.07) in the control. While the lowest amount (6±1) was recorded in the third larvae instar.

Total Coagulocytes at all treated day with tylosin showed that, the control has the highest amount (37±6.06). While at first day the lowest amount of total Coagulocytes (14.33±1.3) was recorded in the fourth larvae instar. After 48 hours the lowest Coagulocytes Without granules amount (2.99±2.365) was recorded in the fourth larvae instar. While at third day the lowest amount (13.34±3.1) was recorded in the second larvae instar.

Spherule at all treated day with tylosin showed that, the control has the highest amount (20.67±9.06). While at first day the lowest amount of Spherule (9.6±1.53) was recorded in the fourth larvae instar. After 48 hours the lowest Spherule amount (10±2) was recorded in the second larvae instar. While at third day the lowest amount (10.67±2.08) was recorded in the third larvae instar.

Oenocytes at the first treated day with tylosin showed that, the third instar has the highest amount (11.33±1.15). As the lowest amount of Oenocytes (5.67±3.21) was recorded in the control. After 48 hours, the fourth instar has the highest amount (14±1.7). The lowest

Oenocytes blood cells amount (5.67±3.21) was recorded in the control. The third day after treated with tylosin showed the highest amount of Oenocytes (12.67±4.04) in the third instar. While the lowest amount ((5.67±3.21) was recorded in the control.

Table 1. effect of Tylosin drug on different honey bee worker blood cells types

Time/ hour	larval Instar	prohaemocytes	Plasmatocytes	Coagulocytes		Total Coagulocytes	Spherule	Oenocytes
				With granules	Without granules			
24	second	25±2.08	18±2.65	8.67±3.5	13.67±1.53	22.34±2.52	16.67±7.5	6.3±2.08
	third	20±5	14.3±4.04	10.33±1.53	14±5.29	24.33±3.41	8.67±4.04	11.33±1.15
	fourth	21.33±1.5	14±2.64	10.33±1.53	4±1	14.33±1.3	9.6±1.53	10±2
	fifth	25±4.62	19±9.6	10±6.7	11±6.93	21±6.8	12±3	7±5.29
48	second	20.67±3.21	16.3±3.79	10±0.00	17.3±0.57	27.3±0.57	10±2	11.67±1.53
	third	24.3±2.31	23.3±4.73	14±3.46	4.67±2.08	18.67±2.77	14.33±3.21	11±2.65
	fourth	25±6.25	17±3.6	12.33±0.57	8.66±4.16	2.99±2.365	11.33±2.89	14±1.7
	fifth	23.3±1.15	20±2	24±2.65	9.33±5.51	33.33±4.08	12.67±6.03	13±4.58
72	second	26±1.7	26.67±3.5	6.67±1.5	6.67±4. 7	13.34±3.1	19±3.46	10.67±2.31
	third	24.3±5.5	21.33±3.05	9.67±2.08	6±1	15.67±1.54	10.67±2.08	12.67±4.04
	fourth	18.67±3.05	16.3±1.53	7.7±0.58	12.67±2.08	20.37±1.33	18±2	11.33±2.08
	fifth	21±6	11.67±6.51	8±2	13.3±10.5	21.3±6.25	11.33±4.9	9±1
	control	13.67±4	26±3	11.67±3.06	25.33±9.07	37±6.06	20.67±9.06	5.67±3.21

Fig (1): showed the effect of treatment time on the blood cells type. The prohaemocytes after 24 hours was increased and reached 22.83±6.6 while the amount in control was 13.67±4. After 48 hour the amount was increased and reached 23.32±3.23. After 72 hours the amount was decreased and reached 22.49±4.06. The Plasmatocytes amount after 24 hours was decreased and reached 16.33±4.73, while the amount in control was 26±3. After 48 hour the amount was increase and reached 19.15±3.53. After 72 hours the amount was decreased and reached 18.99±3.65. All The Plasmatocytes amount reached amount less than control at all time. The Coagulocytes With granules after 24 hours was decreased and reached 9.83±3.32while the amount in control was 11.67±3.06. After 48 hour the amount was increased and reached 15.09±1.67. After 72 hours the amount was decreased and reached 8.01±1.54.

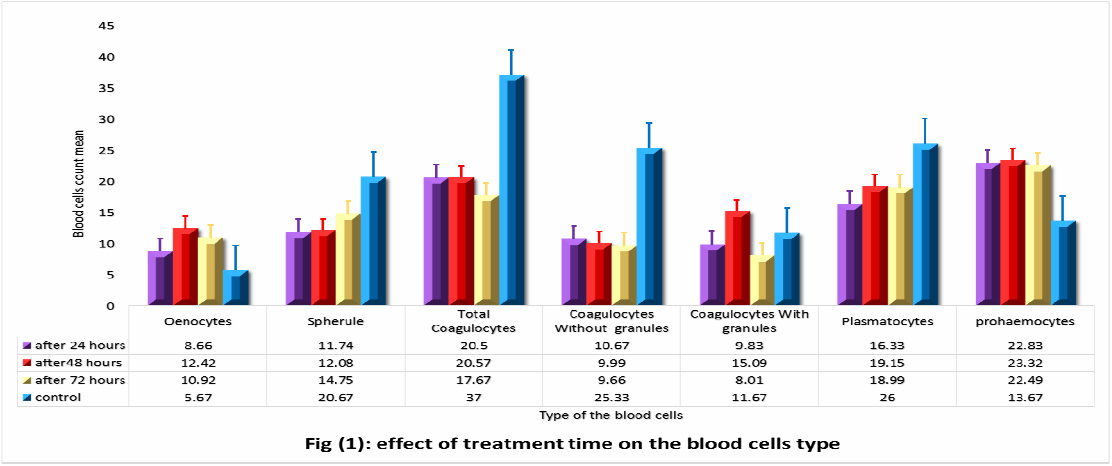
The Coagulocytes Without granules after 24 hours was increased and reached 10.67±3.69 while the amount in control was 25.33±9.07. After 48 hour the amount was increased and reached 9.99±3.06. After 72 hours the amount was slightly decreased and reached 9.66±4.57.

The total Coagulocytes after 24 hours was increased and reached 20.5±3.51 while the amount in control was 37±6.06. After 48 hour the amount was increased and reached 20.57±2.45. After 72 hours the amount was decreased and reached 17.67±3.055.

The Spherule after 24 hours was increased and reached 11.74±4.02 while the amount in control was 20.67±9.06. After 48 hour the amount was increased and

reached 12.08±3.53. 72 hours late the amount was increased and reached 14.75±3.11.

The Oenocytes blood cells after 24 hours was increased and reached 8.66±2.63 while the amount in control was 5.67±3.21. 48 hour late the amount was increased and reached 12.42±2.62. 72 hours late the amount was decreased and reached 10.92±2.36.



	Oenocytes	Spherule	Total Coagulocytes	Coagulocytes Without granules	Coagulocytes With granules	Plasmatocytes	prohaemocytes
after 24 hours	8.66	11.74	20.5	10.67	9.83	16.33	22.83
after48 hours	12.42	12.08	20.57	9.99	15.09	19.15	23.32
after 72 hours	10.92	14.75	17.67	9.66	8.01	18.99	22.49
control	5.67	20.67	37	25.33	11.67	26	13.67

Fig (1): effect of treatment time on the blood cells type

Fig (2): showed the effect of larval age on the blood cells type. The prohaemocytes cells in the second larvae Instar was increased and reached 26±1.7 while the control amount was 13.67±4. As The third larvae Instar amount was decreased and reached 24.3±5.5, while the fourth larvae Instar amount was decreased and reached 18.67±3.05. Whereas the fifth larvae instar amount was decreased and reached 21±6.

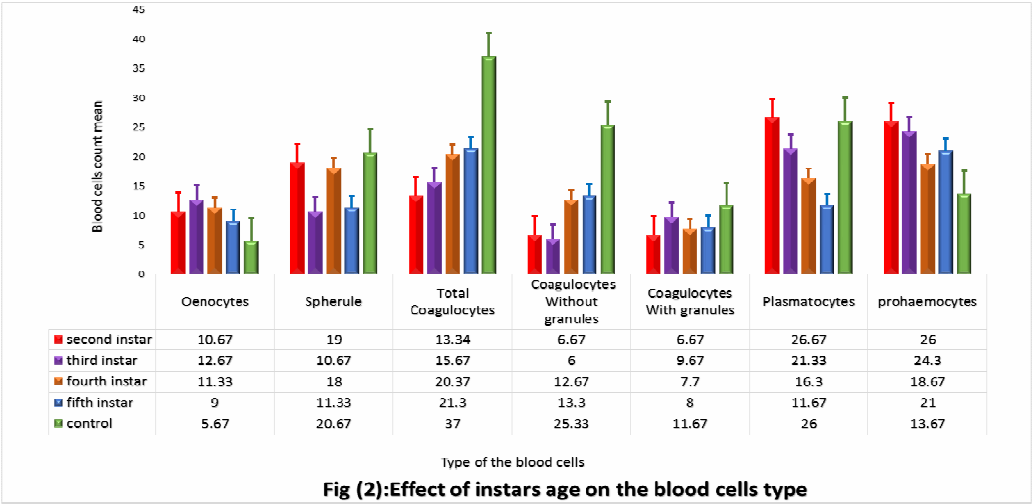
The Plasmatocytes blood cells in the honeybee second larvae Instar was increased and reached 26.67±3.5 while the control amount was 26±3. The third larvae Instar amount was decreased and reached 21.33±3.05. As The fourth larvae Instar amount was decreased and reached 16.3±1.53, while the fifth larvae Instar amount was decreased and reached 11.67±6.51.

The Plasmatocytes cells in the second larvae Instar was increased and reached 26.67±3.5 while the control amount was 26±3. The third larvae Instar amount was decreased and reached 21.33±3.05. As The fourth larvae Instar amount was decreased and reached 16.3±1.53, while the fifth larvae Instar amount was decreased and reached 11.67±6.51.

The Coagulocytes cells with granules in the second larvae Instar was decreased and reached 6.67±1.5 while the control amount was 11.67±3.06. The third larvae Instar amount was increased and reached 9.67±2.08. As The fourth larvae Instar amount was decreased and reached 7.7±0.58, while the fifth larvae Instar amount was increased and reached 8±2.

The Coagulocytes Without granules cells in the second larvae Instar was decreased and reached 6.67±4. 7while the control amount was 25.33±9.07. The third larvae Instar amount was decreased and reached 6±1. As The fourth and fifth larvae Instar amount was increased and reached 12.67±2.08 and13.3±10.5 respectively.

The Total Coagulocytes blood cells in the second larvae Instar was decreased and reached 13.34±3.1. While the control amount was 37±6.06. The third, fourth and fifth larvae Instar amount was increased and reached 15.67±1.54, 20.37±1.33 and 21.3±6.25 respectively.



	Oenocytes	Spherule	Total Coagulocytes	Coagulocytes Without granules	Coagulocytes With granules	Plasmatocytes	prohaemocytes
second instar	10.67	19	13.34	6.67	6.67	26.67	26
third instar	12.67	10.67	15.67	6	9.67	21.33	24.3
fourth instar	11.33	18	20.37	12.67	7.7	16.3	18.67
fifth instar	9	11.33	21.3	13.3	8	11.67	21
control	5.67	20.67	37	25.33	11.67	26	13.67

Fig (2):Effect of instars age on the blood cells type

The Spherule cells in the second larvae Instar were decreased and reached 19±3.46 while the control amount was 20.67±9.06. The third larvae Instar amount was decreased and reached 10.67±2.08. As The fourth larvae

Instar amount was increased and reached 18±2, while the fifth larvae Instar amount was decreased and reached 11.33±4.9.

The Oenocytes cells in the second larvae Instar were increased and reached 10.67±2.31 while the control amount was 5.67±3.21. The third larvae Instar amount was increased also and reached 12.67±4.04. As The fourth larvae Instar amount was decreased and reached 11.33±2.08, while the fifth larvae Instar amount was decreased and reached 9±1.

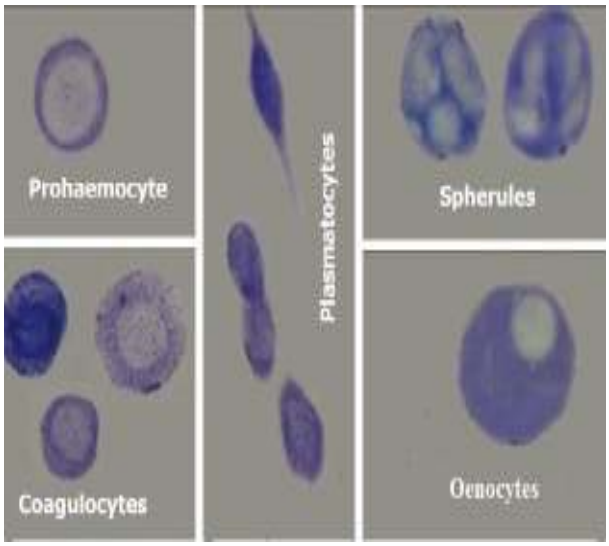


Fig 3. blood cells type

From fig., (4) which represented the instar blood cells different between treatment and control. It could be concluded that, the prohaemocytes and the oenocytes count cells was increased more than the control. But the Plasmatocytes, Coagulocytes with granules, Coagulocytes without granules, total Coagulocytes, and spherule count cells was decreased more than the control in 24, 48, and 72 hours.. In generally the blood cells account were declined except in prohaemocytes and oenocytes, this finding strongly suggested that, the tylosin drug time treatment has a negative effect on the most blood cells count, this result powerfully recommended that, the tylosin drug instar treatment has a negative effect on the most blood cells count.

The results in fig., (5) showed the instar blood cells different between treatment and control. It could be concluded that, the prohaemocytes and the oenocytes cells count was increased more than the control. But the Plasmatocytes, Coagulocytes with granules, Coagulocytes without granules, total Coagulocytes, and spherule count cells was decreased more than the control in second, third, fourth and fifth larvae instar. In mostly the blood cells mount were decreased except in prohaemocytes and oenocytes, this finding strongly suggested that, the tylosin drug larvae instars have a negative effect on the most blood cells count.

No much more researchers had discussed the tylosin effect on the honeybee workers larvae blood cells, maybe this is the first paper research thus discussed the tylosin effect on the workers larvae blood cells. Most of all tylosin paper research had attempts, in the antimicrobial and the residue effect and found that, Tylosin exerts bacteriostatic effect on Mycoplasma species and Gram-positive bacteria. It is well absorbed when administered by oral and parenteral routes and is eliminated from the body slowly. Tylosin binds to serum and milk proteins at rates of 25–47% and 15%, respectively (Liu M *et al*., 2002 and Bush *et al*., 2001). In most animal species, the half-life (t1/2) of tylosin is 3-4 h and the volume of distribution (v d) ranges between 1 and 7 L/kg. Tylosin passes readily into milk and its concentrations in milk may be five times higher than its plasma concentrations. Tylosin does not undergo major modification in the body and is excreted mainly in bile and milk and partly in urine (Kolpin *et al*., 2002). The unmodified form of tylosin passes into milk and eggs.

As reported by the European Medicines Evaluation Agency (EMEA), the maximum residue limit (MRL) established for tylosin in cow's milk is 50 g/kg. The with drawl periods for meat and milk, in the event of the parenteral administration of tylosin to cattle, sheep, and goats, are 28 days and 8 milking, respectively (Teeter *et al*., 2003).

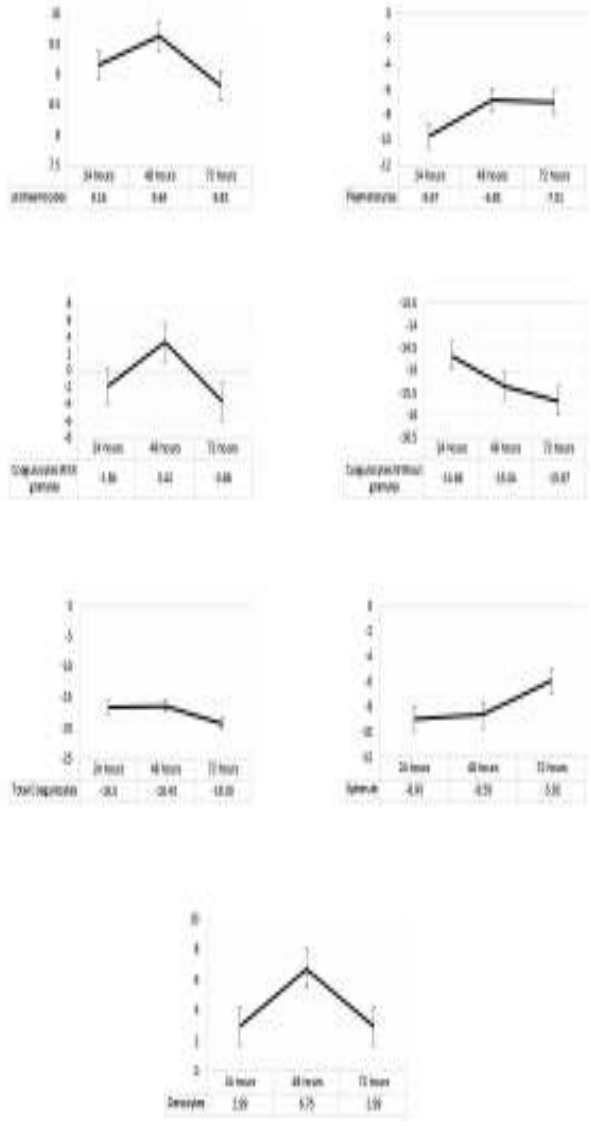


Fig 4. The blood cells different between treatment and control correspond to the time

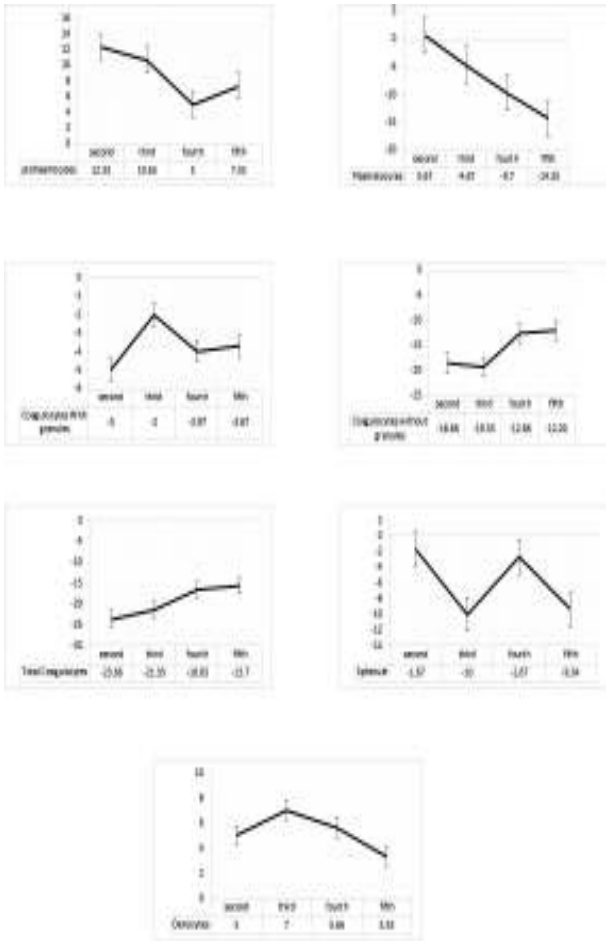


Fig 5. blood cells different between treatment and control correspond to the larvae instar

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تأثير عقار التيلوزين على خلايا دم يرقات شغالات نحل العسل

أشرف شريف فتحي شريف

مركز البحوث الزراعية معهد وقاية النبات قسم بحوث النحل

يعد عقار التيلوزين من أشهر مضادات الجراثيم التي تنتجها البكتريا والمستخدمة في مجال تربية نحل العسل وخاصة لعلاج أمراض تعفن الحضنة. الهدف الرئيسي من هذه الدراسة هو دراسة تأثير عقار التيلوزين على أعداد/أنواع خلايا الدم المختلفة في يرقات نحل العسل. فمن المعروف ان المناعة في الحشرات تعتمد على أنواع معينة من خلايا الدم. عوملت خلايا النحل بمحلول يحتوي على التيلوزين ثم أخذت عينات من دم اليرقات وفحصت بالمجهر بعدسة زيتية بقوة 100x. أظهرت النتائج انخفاض في عدد خلايا الدم ماعدا نوعين الخلايا بروهيموسيتس وأونوسيتس في كلا متغيري التجربة (الوقت والعمر اليرقي). توصي النتائج بتقليل استخدام عقار التيلوزين لما يسببه من تأثير سلبي على بعض خلايا الدم مما قد يؤدي لانخفاض المناعة.