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Research Article

Influence of Ecdysone Agonist, Chromafenozide on Haemocytes of Spodoptera Mauritia (Boisd) (Lepidoptera: Noctuidae)

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ABSTRACT

The rice swarming caterpillar, *Spodoptera mauritia*, is one of the major paddy pest, which causes serious loss of rice crops. Several problems associated with the use of conventional insecticides have strongly demonstrated the need for applying alternative safe compounds such as insect growth regulators (IGRs). The present study was carried out to investigate the effect of ecdysone agonist, Chromafenozide on the larval hemogram of *S. mauritia*. The fifth instar (penultimate) day 0 larvae were treated with three known concentrations of Chromofenozide(0.5µM, 1µM,2µM) and they were observed at 24 hour intervals. THC was found to first increase and then decrease at lower concentrations, while at higher concentration, it was found gradually decreasing. The haemogram evidenced after the treatment with lower concentrations showed a remarkable decrease in PLs, OEs, PRs ADs, POs, and VEs at 24 and48 hours, while GRs and SPs exhibited an increase than the control. At higher concentrations, THC values of haemocytes were found to be decreased after 24 and 48 hours as when compared to control. Also, the percentage of all the haemocytes decreased after 72 hours of treatment with three concentrations, as when compared with the control. Administrations of these three concentrations resulted in various cellular abnormalities. The impact of ecdysone agonist, chromafenozide exhibited a decrease in capability of larval immune defense of *S.mauritia*, by altering the hormonal triggers in the treated individuals, causing significant changes both quantitatively and qualitatively in the haemogram obtained.

Keywords: Haemocytes, *Spodoptera mauritia*, IGRs, Chromafenozide, Cellular immunity, Haemogram.

INTRODUCTION

Agriculture is considered as one of the demographically broadest economic sector and plays a significant role in overall socioeconomic status of India. But, it should be noted that Indian agricultural system is always at the threat of various factors via, changes in climatic conditions, physical parameters of environment and damages caused by insect pests and other enemies. In order to reduce the pest attack, different pesticides, which covers a wide range of compounds including synthetic organic compounds; insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others are widely used by farmers for pest control and agricultural output. However, the use of many cocktails of pesticide had resulted adverse effects to the environment and to the mankind. To reduce the negative impact of pesticides to the environment and to the human beings, the concept of insect pest management programme was developed, which eventually were widely practiced.

Insect growth regulators (IGR) are considered as substances that disrupt the normal activity of the endocrine/hormone system of insects, affecting the development, reproduction, or metamorphosis of the target insects. Several classes of IGRs, such as juvenile hormone analogues/anti- JH compounds, ecdysone mimics and chitin synthesis inhibitors cause a wide range of developmental derangements in susceptible species, affecting embryogenesis, larval development, metamorphosis and reproduction. Ecdysone is a major hormone regulating the metamorphosis of insects, so they have become one of the target hormones in IGR research. In fact, a number of chemicals have been developed depending on the mode of action of ecdysone in insect's body as ecdysone agonists and antagonists. Many of these compounds belong to the class of bisacylhydrazines, that are highly specific to lepidopteran pests and induces precocious lethal moult[1].

Insect immune system is known to involve both cellular and humoral factors which together form a potent defence against invading organisms[2]. The cellular immune responses are functionally including phagocytosis, nodule formation and encapsulation[3] referred to as hemocyte-mediated processes. Haemocyte Science is very vast, evergreen and interesting subject for scientific community. The ability to isolate and identify haemocytes is essential for studies in insect cellular immunity. The armyworm, *Spodoptera mauritia* Boisduval is considered to be a sporadic pest which occasionally causes serious losses to rice crop. The effect of insecticides on haematological study and haemocyte morphology has been studied only in few insect species. Hence, the present study an attempt has been made to determine changes in the haemocyte count, after treatment with an edysone agonist, chromafenozide, on fifth instar larvae of *Spodoptera mauritia.*

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MATERIALS AND METHODS

Collection and maintenance of insect culture

The adult moths of *S. mauritia* were collected at night using fluorescent lights. They were kept in glass beakers covered with muslin cloth and were fed with a dilute solution of honey and maintained at laboratory conditions of 28±2 ^{0}C, RH 90±30% under 12 hour light: 12 hour dark photoperiod. They were allowed to lay eggs on the cloth.Larvae hatched out after 3-4 days.The larvae were reared in glass chimney and were fed with fresh leaves of young paddy plants or leaves of the grass *Ischaemum aristatum*. When the larvae grew in size, they were kept in large plastic troughs with enough space for free movement. The total larval period was found to range from 17 to 19 days and consisted of 6 larval instars.

Chemical compound

The non-steroidal ecdysone agonist chromafenozide, (2'-tert-butyl-5-methyl-2'-(3,5-xyloyl)-chromane-6-carbohydrazide) purchased from Sigma Aldrich were used for the study. The compound was dissolved in acetone and diluted to obtain the required concentrations.

Treatments

Newly moulted fifth larval instars (day0) were segregated from the stock colony in glass petridish. Three sub lethal concentrations of chromafenozide; 0.5µM,1µM and 2µM/5µl were taken for the experimental procedure. The concentrations were prepared by dissolving the IGR in solution of acetone to get the appropriate concentration. Hamilton micro syringe was used for treatment. Control larvae were treated with an equivalent amount of acetone. Topical application was used for the treatments on larvae, by using Hamilton micro syringe (0-10µl).The treated and control larvae were kept in separate beakers.

Smear preparation and Staining

Spodoptera mauritia haemolymph samples were obtained from the desired stage. A drop of fresh haemolymph was collected by puncturing proleg on the abdominal segment of the larva (with the help of 70% ethanol sterilized needle) on a slide and mixed well with anticoagulant. A thin uniform smear of haemolymph was spread on the slide by rubbing the edge of an inclined slide backward. Stock solution of Giemsa stain was prepared as per protocol (Tauber and Yeager, 1935). A portion of it was diluted 10 times with double distilled water (DDW). Then air dried smear was stained for 20 min and thereafter rinsed with DDW and mounted in DPX.

Differential and Total Haemocyte Count (DHC and THC)

DHC was conducted by counting different categories of randomly selected cells from stained smears of 10 individuals.For THC, the haemolymph was drawn in to a thoma blood cell pipette up to its graduated mark of 0.5 and diluted up to the 11th mark with Tauber-Yeager's fluid(Tauber and Yeager, 1935), then shaken for several minutes and the first three drops were discarded. A double line with improved Neubauer ruling haemocytometer was filled with diluted haemolymph and the haemocytes were counted at four corners and one central (1mm^2) square. When the distribution of cells in all squares was not even, the sample was discarded and the procedure was repeated. The number of haemocytes per cubic millimeter (mm^3) was calculated using the formula of Jones (1962):

$$\frac{\text{Haemocytes in five } 1\text{mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted.}}$$

Where dilution =22 times, depth factor of the chamber = 10(constant) and the number of square counted =5.

Statistical analysis

Mean± Standard deviation (SD) and Standard error (SE) were calculated by using the SPSS program version 16.00.

RESULTS

The fifth instar (penultimate) day 0 larvae, treated with three known concentrations (0.5µM, 1µM, 2µM), produced tremendous effects on morphology as well as in haemocyte physiology.

Quantitative analysis of Total Haemocyte Count (THC)

The results of quantitative haemolymph profile of day1, day2 and day3 fifth instar larvae were obtained as- (a) day1 larvae=1.7600±27.438 cells/mm^3, (b) in day 2 larvae= 1.9140±20.871 cells/mm^3 and (c) day 3 larvae = 2.002± 33.525 cells/mm^3 (Table 1).

Effect of chromafenozide on the THC of fifth instar larvae

The effect of treatment after the topical application of chromafenozide was assessed on hematological parameters by THC after 24, 48 and 72 hours.

Topical treatment with 0.5µM and1µM concentration caused a dose dependent increase in THC after 24 and 48 hours. When compared to untreated larvae (1.7600±27.438cell/mm^3 of haemolymph), the mean value of THC progressively increased to (2.112±15.382cells/mm^3 and 2.002±33.525 cells/mm^3 of haemolymph) at 24 hour treatment of the compound with 0.5 and 1µM respectively. After 48 hours the values of THC reached 2.260±23.303cells/mm^3 and 2.1780±16.2303cells/mm^3 as compared to 1.9140±20.871 cells/mm^3 in control with 0.5µM and 1 µM concentration respectively.

These concentrations also caused a dose dependent decrease in the THC after 72 hour treatment. THC reached (1.892±34.707cells/mm^{3}and 1.276±38.526 cells/mm^3) as when compared to 2.002±33.525cells/mm^3 in control after 72 hour.

A significant decline in the total number of circulating haemocytes was recorded after 24, 48 and 72hours of post treatment of larvae with 2µM chromafenozide. The mean values of THC were 1.276±38.526cells/mm^3,1.099±18.441 cells/mm^3,1.051±18.199 cells/mm^3 at 24, 48 and 72 hours respectively. (Table 1)

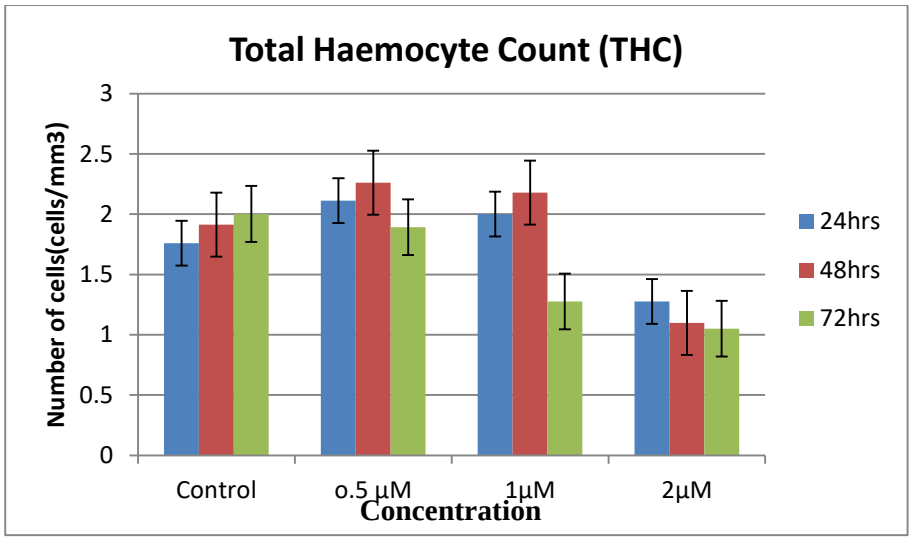
Quantitative analysis of differential haemocyte count (DHC) of fifth instar larvae

The DHC was done with the objective to find out the number of various classes of haemocytes in the heamolymph of 5th instar day 1, 2 and 3 larvae. The results revealed that the number of Granulocytes were high (260±3.740), followed by Plasmatocytes, Pl(205.40±3.333), Spherulocytes, SP(43.30±3.162), Oenocytoids, OE(25.3±1.316), Prohaemocyte, PR(18.19±2.131), Adipocytes, AD(14.0±1.054),

Table 1: Total haemocyte count (thc) of *spodoptera mauritia* at 24hr,48hrand 72 hr after chromafenozide treatment.

	Control	o.5 µM	1µM	2µM
24hrs	1.7600 ± 27.438 (8.676)	2.112 ± 15.382 (4.864)	2.002± 33.525 (10.601)	1.276±38.526 (12.183)
48hrs	1.9140 ± 20.871 (6.600)	2.260 ± 16.233 (5.133)	2.1780±16.230(6.833)	1.099± 18.441 (4.831)
72hrs	2.002 ± 33.525 (10.601)	1.892 ±34.707 (10.975)	1.276±38.526 (12.183)	1.051 ± 18.199 (5.755)

Mean ± standard deviation (standard error), n=10



podocytes, PO(13.26±1.349) and vermicytes, VE(12.44±2.875) in day 1 larvae. (Table 2)

In day 2 larvae the number of granulocytes were slightly declining (139.32±6.408), followed by plasmatocytes (115.18±5.370), Prohaemocytes (37.27±5.334), Adipocytes(22.21±8.685),Spherulocytes(20.85±4.836),Podocytes (16.79±4.976), Oenocytoids (15.11±5.384) and Vermicytes(10.3±5.981). (Table 3)

In day 3 larvae the number of granulocytes were decreasing (118.46±5.501), with respect to Plasmatocytes (100.24±6.040), Prohaemocytes (27.77±6.254), Spherulocytes (20.84±3.921), Adipocytes (17.73±2.540), Oenocytocytes (14.16±3.438),Vermicytes (7.27±3.888) and Podocytes (4.94±3.533). (Table 4)

Effect of chromafenozide on the DHC of fifth instar larvae

From the haemogram evidenced after 24 hours treatment of chromafenozide, the percentage of PLs, OEs, PRs ADs, POs, and VEs was found to decrease as 191.9±49.758, 22.09±5.586, 14.5±7.546, 12.04±5.146, 8.46±5.378 and 8.71±6.118, with 0.5 µM concentration and 178.1±5.033, 22.40±3.559, 11.97±3.465, 11.54±6.670, 9.04±3.234 and 8.07±4.001 with 1µM concentration respectively (Table 2).

After 48 hours of chemical treatment the percentage of PLs, Ads, OEs, POs, SPs and VEs decreased from 105.37±6.650, 20.19±4.332, 17.76±5.059, 16.48±5.633, 10.20±4.853 and 4.97±3.772 to 101.5 ±6.912, 12.20±4.320, 14.08±3.084, 14.02±4.184, 13.75±5.854 and 3.58±5.533 with the application of treatment with 0.5 and 1µM respectively. The GRs and PRs increased from 228.6±6.614 and 45.34±4.526 then declined to 177.5±7.530and 40.16± 5.758 respectively with the same concentrations (Table 3).

Significant decline in the population of haemocytes were observed after the topical application of compound (2 µM) and the values of PLs, GRs, PRs, SPs, POs, VEs, OEs, and Ads decreased after 24 and 48 hours as when compared to control. However, the percentage of all the haemocytes decreased after 72 hours treatment with all the three concentrations as when compared with the control as shown in (Table 4).

Effect of chromafenozide on haemocyte morphology

After the application of 0.5µM concentration of chromafenozide, major changes are observed in granulocytes and plasmatocytes. The other notable changes are seen in granulocytes having ruptured cell wall, with scattered nucleus, few granulocyte with cytoplasmic projection, disintegrated cell wall of spherulocyte, loss of cytoplasmic compactness of pseudopods of plasmatocytes and oenocytoids with abnormal cytoplasmic extension(Figs :A-F).

The observed abnormalities after administration of 1µM concentration, are the following; Expansion of vermicytes, loss of compactness of pseudopods of plasmatocytes , few of them with dispersed cell contents and adipocytes without cell wall and loss of cytoplasmic contents of granulocyte (Figs :B-F).

Examination of haemocyte profile, after the administration of 2µM chromafenozide, revealed significant changes including abnormal staining of cells, Spherulocytes with damaged spherules, lobed nucleus in plasmatocytes and with a cytoplasmic projection, distortion of the shape of haemocytes especially granulocytes and plasmatocytes. Clumping of haemocytes was another predominant features followed by rupturing of the wall of haemocytes (Figs :A-C).

Table 2: Effect of chromafenozide after 24 hr treatment on differential haemocyte count (dhc) of 5th larval instar of *spodoptera mauritia* at successive concentrations.

Haemocyte type	Control	0.5µM	1µM	2µM
Plasmatocytes	205.40±3.333 (1.054)	191.9±49.758 (15.735)	178.1±5.033(1.591)	161.28±4.756 (1.504)
Granulocytes	260±3.740 (1.185)	283.4±7.546 (2.386)	274.3±4.332 (1.369)	231.10±5.223 (1.651)
prohaemocytes	18.19±2.131 (0.674)	14.5±7.152 (2.261)	11.97±3.465 (1.095)	11.13±3.560 (1.125)
spherulocytes	43.30±3.162 (1.0)	43.52±6.390 (2.021)	45.06±4.221 (1.335)	42.06±3.717 (1.175)
podocytes	13.26±1.349 (0.426)	8.46±5.378 (1.700)	9.04±3.234 (1.024)	5.32±5.181 (1.443)
vermicytes	12.44±2.875 (0.909)	8.71±6.118 (1.934)	8.07±4.001 (1.265)	5.78±5.181 (1.638)
oenocytoids	25.3±1.316 (0.416)	22.09±5.586 (1.766)	22.40±3.559 (1.125)	21.41±4.886 (1.545)
Adipocytes	14.0±1.054 (0.333	12.04±5.146 (1.627)	11.54±6.670 (2.109)	6.41±3.813 (1.206)

Mean±Standard deviation (Standard error), n=10

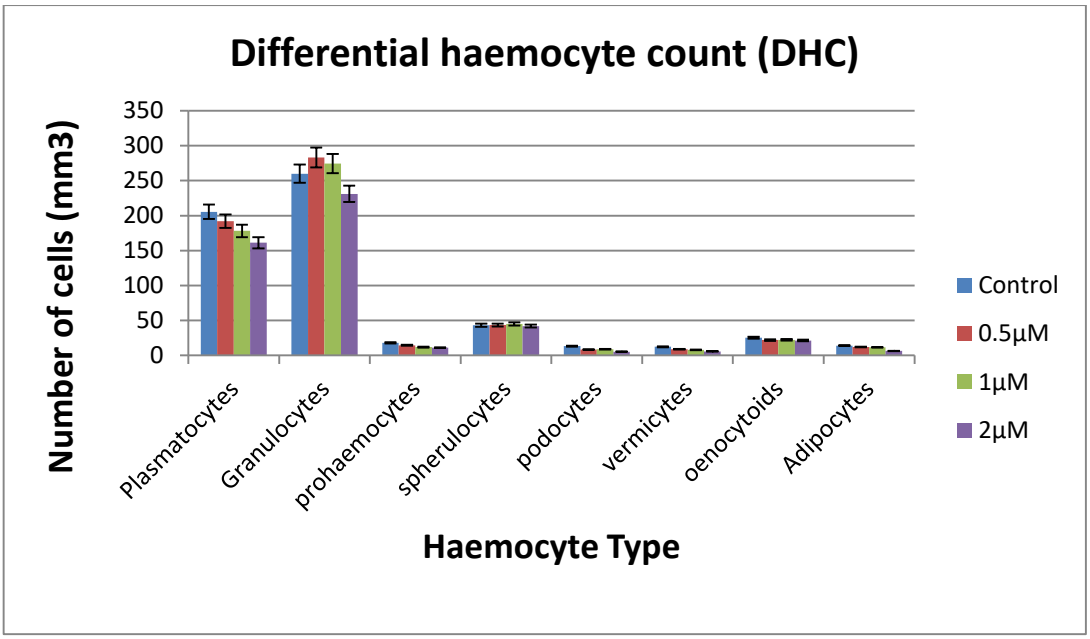


Table 3 : Effect of chromafenozide after 48 hr treatment on differential successive concentrations.

Haemocyte type	Control	0.5µM	1µM	2µM
Plasmatocytes	115.18±5.370 (1.698)	105.37±6.650 (2.103)	101.5±6.912 (2.185)	94.11±3.270 (1.090)
Granulocytes	139.32±6.408 (2.026)	228.6±6.614 (2.092)	177.5±7.530 (2.381)	126.1±4.387 (1.462)
prohaemocytes	37.27±5.334 (1.686)	45.34±4.526 (1.431)	40.16±5.758 (1.820)	36.02±4.969 (1.656)
spherulocytes	20.85±4.836 (1.529)	10.20±4.853 (1.534)	13.75±5.854 (1.851)	7.13±4.795 (1.598)
podocytes	16.79±4.976 (1.573)	16.48±5.633 (1.781)	14.02±4.184 (1.323)	7.0±4.795 (1.598)
vermicytes	10.3±5.981 (1.891)	4.97±3.772 (1.193)	3.58±5.533 (1.749)	2.55±5.650 (1.886)
oenocytoids	15.11±5.384 (1.702)	17.76±5.059 (1.600)	14.08±3.084 (0.975)	8.12±4.969 (1.656)
Adipocytes	22.21±8.685 (2.746)	20.19±4.332 (1.369)	12.20±4.320 (1.366)	11.13±4.358 (1.452)

Mean±Standard deviation (Standard error), n=10.

From these observations it is clear, that the ecdysone agonist chromafenozide mainly affected the granulocytes and plasmatocytes population.

DISCUSSION

Gupta studied the haemocytes present in Lepidoptera, Hymenoptera, Coleoptera and Diptera. Later, few researchers focused on free haemocytes of Lepidopteran insects using different types of insecticides, toxins etc[4]. The information on the recently developed ecdysone agonist chromafenozide on *Spodoptera mauritia* is lacking, so the present study aimed to elucidate the effect of chromafenozide on the haemocytes of rice swarming caterpillar. The observations obtained are in accordance with the results obtained by[5,6]. Earlier studies of Smagghe and Degheele observed similar effect on larval stages of a number of lepidoptera species after the application of tebufenozide (RH-5992)[7].

THC has been found to be quite variable depending upon the insect species, developmental stage, physiological state and the technique followed[8]. This is the first study of the kind to examine the major types of haemocytes and also to demonstrate the effect of ecdysone agonist, chromafenozide on the haemogram of fifth instar larvae of *Spodoptera mauritia*. The increase in THC by chromafenozide may be correlated to the degree of the

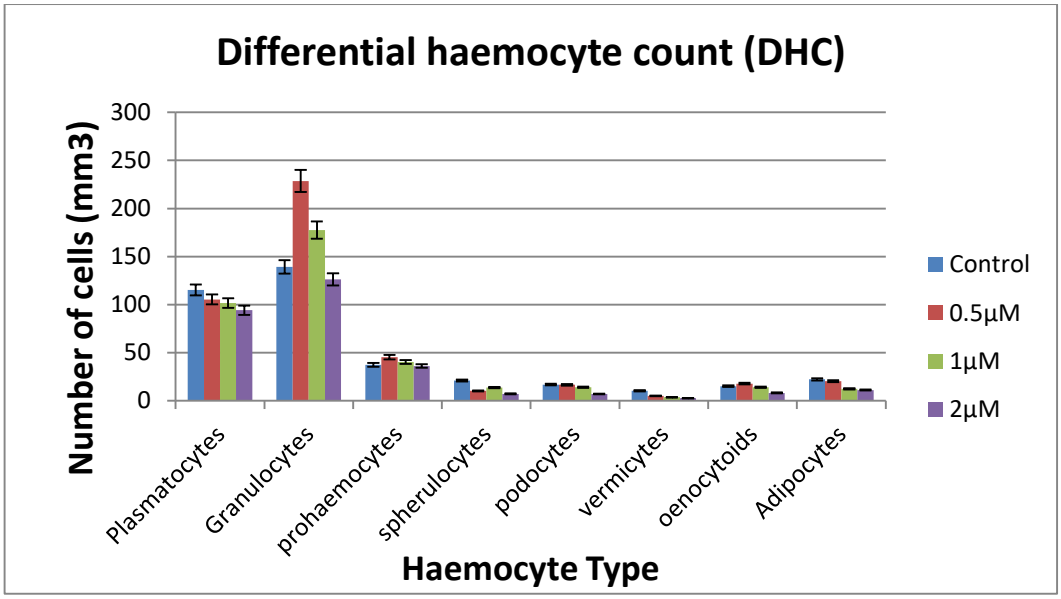
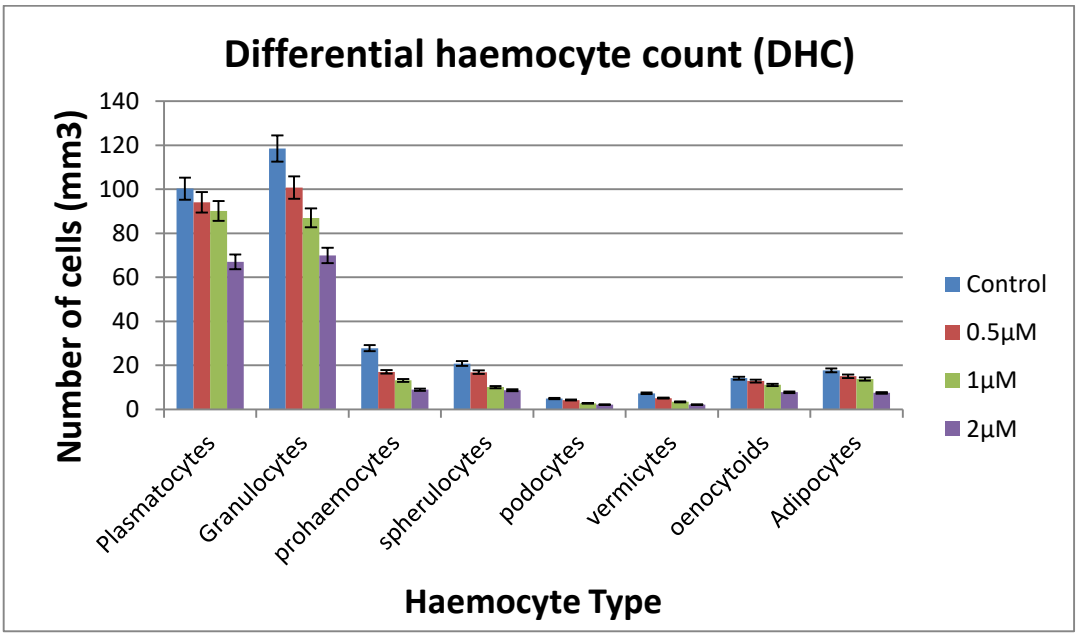


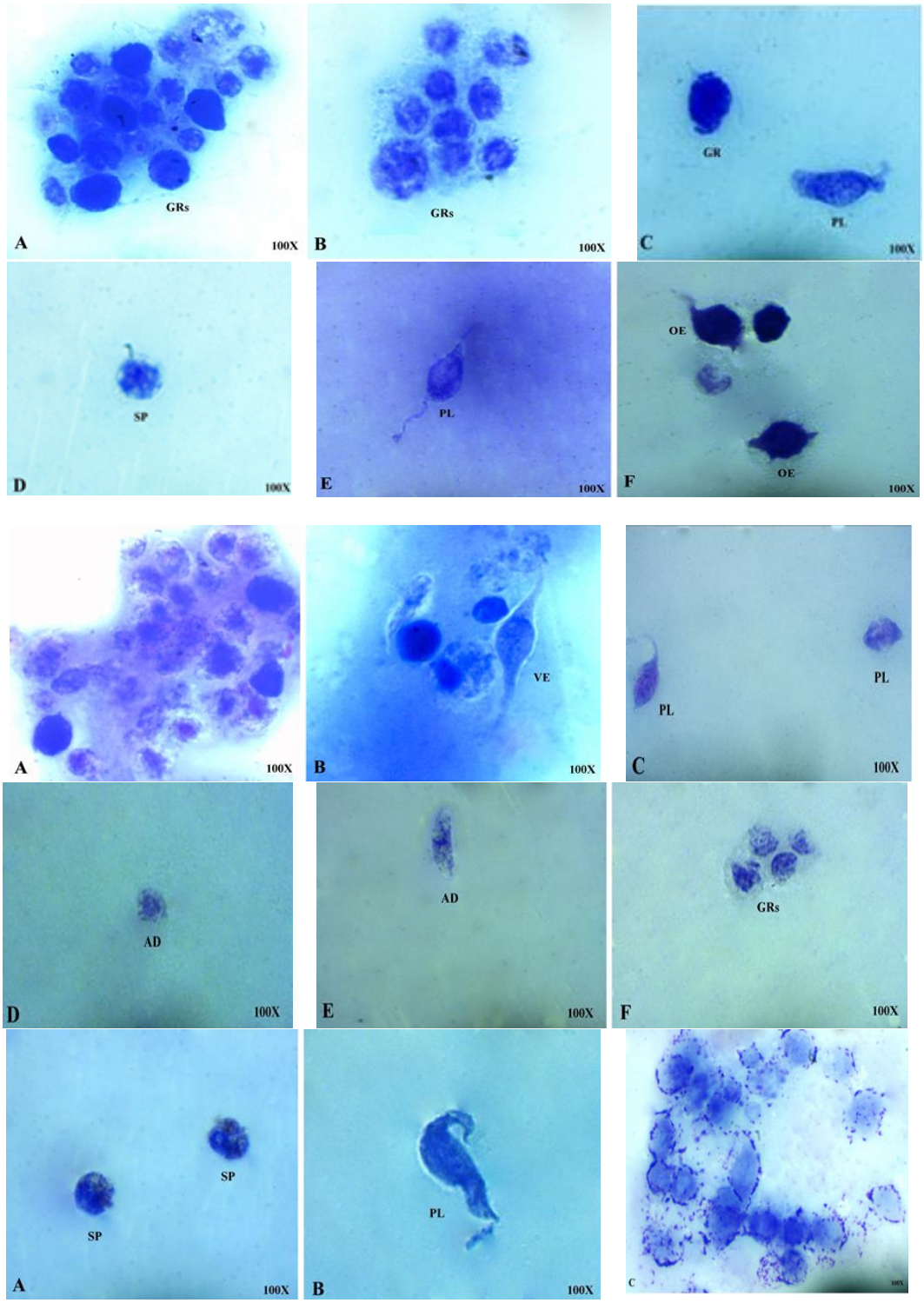
Table 4 : Effect of chromafenozide after 72 hr treatment on differential haemocyte count (dhc) of 5th larval instar of *spodoptera mauritia* at successive concentrations.

Haemocyte type	Control	0.5µ	1µ	2µ
Plasmatocytes	100.24±6.040 (1.910)	94.06±3.470 (1.097)	90.06±3.864 (1.222)	67.03±3.973 (1.256)
Granulocytes	118.46±5.501 (1.739)	100.73±2.451 (0.775)	86.99±3.446 (1.089)	69.90±3.887 (1.229)
prohaemocytes	27.77±6.254 (1.977)	17.05±4.326 (1.368)	13.18±3.765 (1.190)	8.93±4.989 (1.577)
spherulocytes	20.84±3.921 (1.240)	16.90±4.055 (1.282)	10.09±31.448 (9.944)	8.75±4.352 (1.376)
podocytes	4.94±3.533 (1.117)	4.22±4.638 (1.466)	2.71±6.244 (1.974)	2.20±1.549 (0.489)
vermicytes	7.27±3.888 (1.229)	5.12±4.685 (1.481)	3.41±4.094 (1.294)	2.14±3.747 (1.185)
oenocytoids	14.16±3.438 (1.087)	12.90±2.213 (0.700)	11.13±2.311 (0.731)	7.79±4.863 (1.538)
Adipocytes	17.73±2.540 (0.803)	15.05±5.016 (1.586)	13.81±5.724 (1.810)	7.50±4.830 (1.527)

Mean±Standard deviation (Standard error), n=10



defensive action of haemocytes involved in detoxification[9]. The increase in the THC after treatment may be due to the release of sessile haemocytes and the activation of mitotic division of the haemocytes, which might be activated in response to some insecticides or IGRs, or it can be suggested as an immune response against pathogen or any foreign body, such as the introduced insecticide[10,11]. The decrease in the number of haemocytes in the treated larvae may be due to nodulation



and encapsulation as well as degranulation of some cell types or the inhibition of the brain hormone secretion[12]. The application of methoprene to the silkworm larvae kept the haemocyte level stable without an obvious change[13]. Recently, Suhail *et al* observed the level of THC in *Coccinella septempunctata* after treatment with spinosad predominantly increased[14].

Prohaemocytes are progenitor stem cells which can differentiate into other types of haemocytes according to light and electron microscopy observations[15]. Chromafenozide exerted a predominant increase in the population of PRs after 48 hrs with lower concentration and reduced after 72 hrs with all concentration. Bhagawathi and Mahantha, recorded a reduction in percentage of PRs with larvae of Eri silk worm against dimethoate[16]. These findings are similar to the results of Bhagawati.N and Mahanta.R (2012)[16] against the application of oraganophosphorus insecticides. In the present study the number of OEs decreased with the increasing concentration of Chromafenozide and exposure

of time. These findings are in accordance with those of Abdel-Aziz *et al*[17] in *S.littoralis* by the exposure of teflubenzuron, Al-Hariri and Suhai,[18] in *S.gregaria* by lambdacyhalothrin and deltamethrin.

The topical application of chromafenozide caused great abnormalities to the blood cells. PLs and GRs were mainly affected cell types by the treatment of chromafenozide. The observations corroborate the findings of Peter and Ananthakrishnan[19] in *Crambe tartarica* with azadirachtin and Sharma *et al* [20]in *S.littura* with *Acorus calamus* oil. The other notable distortions are change in the shape of haemocytes, such as rupturing wall of the cells, abnormal staining of cells.

CONCLUSION

In conclusion, the impact of ecdysone agonist, chromafenozide exhibited a decrease in capability of larval immune defense of *S.mauritia*, by altering the hormonal triggers in the treated individuals, causing significant changes both quantitatively and qualitatively in the haemogram obtained. The sub lethal concentrations of chromafenozide could strongly interfere with the differential and total haemocyte count, and there by decrease the capability of larval immune defense. They showed the ability to make abnormalities in morphology as well as in haemocyte physiology, so there by affecting the survival of *Spodoptera mauritia*.

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