

## Differential haemocyte counts of 5<sup>th</sup> instar nymphs and adults of *Dysdercus cingulatus* Fabr (Hemiptera: Pyrrhocoridae) treated with acephate, an organophosphorus insecticide

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### Abstract

Five types of haemocytes have been identified in the haemolymph of 5<sup>th</sup> instar nymphs and adults of the red cotton bug, *Dysdercus cingulatus*. Changes in the Differential Haemocyte Counts (DHCs) have been assessed in relation to application of graded concentrations of Acephate. The haemogram profile was determined 6 hr, 1 day, 3 days, 5 days post-treatment as well as post-moulting i.e. in the adult males and females. Different types of haemocytes registered a dose-dependent response by either exhibiting increase or decrease in their relative proportions. The adipohaemocytes were the most sensitive cells to the insecticidal stress whereas the oenocytoids showed least damage to their cellular integrity. However, there was a consistent increase in the proportion of damage/unidentifiable blood cells in accordance with increase in concentration of acephate applied. Furthermore, the treated insects apparently responded by releasing more prohaemocytes, the so called "stem cells", into the circulation as evident by increase in the percentage of these cells in treated blood smears compared to the parallel control.

**Keywords:** DHC, prohaemocytes, plasmatocytes, adipohaemocytes, granulocytes, oenocytoides, organophosphate insecticide.

### Introduction

The haemocytes perform various physiological functions in the body of insect. They direct nutrients to various tissues and store them. They perform phagocytosis, encapsulation of foreign bodies in the insect body cavity, coagulation to prevent loss of blood, nodule formation, transport of food materials, hormones and detoxification of metabolites and biological active materials (Patton, 1961). Haemocytes migrate towards and engulf several targets such as apoptotic bodies, cell debris from damaged tissues and pathogens (Wood and Jasinto 2007). Jones (1964) suggested that haemocytes of insects are comparable in their morphology, embryonic origin, amoeboid movements and phagocytic activity to white blood cells of mammals. Knowledge of normal haemocytes of an insect is necessary to physiologists, toxicologists and biochemists, as alterations in structure, types and number of cells reflects changes in physiological and biochemical processes. Insect haemocytes respond to internal changes during development (at ecdysis) and to conditions such as starvation, wounding, parasitism, diseases, chemicals including insecticides. In the present work, an attempt has been made to determine the changes appearing in the population of different haemocytes after topical treatment of 5<sup>th</sup> instar nymphs of Red Cotton Bug, *Dysdercus*

*cingulatus* with Acephate (an organophosphate insecticide).

### Materials and Methods

DHCs were recorded in the permanent stained smears. One blood smear was made from an individual insect and for each treated and control group five smears were observed. About 100-200 cells were counted and categorized in each smear. The differential haemocyte count was calculated in terms of percentage of different types of haemocytes in different observations. The relative percentage of prohaemocytes, plasmatocytes, adipohaemocytes, granulocytes and oenocytoids of affected nymphs were determined 6 hrs, 1 day, 3 days and 5 days following the treatment with all the selected concentrations of acephate. Moreover, the relative population of damaged/unidentified cells was also calculated. When the treated nymphs moulted to adult stage, DHC was ascertained in one-day-old adult males and females. The DHCs of treated nymphs and affected adults were compared with those of the control insects of corresponding age and stage. To measure the intensity of association between DHC and concentrations of chemicals, a regression line was fitted to the data followed by correlation analysis (Sokal and Rohlf 1981).

## Results and Discussion

Six hours after application of acephate the percentage of prohaemocytes exhibited a linear increase with increase in concentration ( $Y=12.37 + 2636.89 X$ ,  $r = 0.9399$ ,  $P<0.001$ ) showing a positive correlation coefficient. With the highest concentration (0.006%), the population of prohaemocytes became nearly three times of the control. The plasmatocytes constituted 53.2% of haemocytes in control nymphs, which subsequently exhibited a negative linear correlation with increase in concentration of acephate applied ( $Y = 48.80 - 6741.72 X$ ,  $r = -0.9779$ ,  $P<0.001$ ). The adipohaemocytes in the normal and acetone treated nymphs of corresponding age constituted 16.58% and 17.06% of total haemocytes. Following the application of graded concentrations (0.001, 0.002 and 0.004%), their relative occurrence was 15.84%, 11.32% and 9.72% respectively. However, in the nymphs affected with the highest concentration (0.006%) these haemocytes were either completely absent or completely damaged showing only the remains of cytoplasm and nuclei in the smear. The density of granulocytes in smears obtained from nymphs, which were affected with 0.001, 0.002 and 0.004% acephate, decreased linearly with increase in concentrations applied ( $Y = 10.57 - 827.24 X$ ,  $r = -0.9884$ ,  $P<0.001$ ). The oenocytoids were relatively few in number in normal and acetone treated insects. In control, the mean percentage of oenocytoids was 3.15 which increased linearly with increase in concentration of acephate applied ( $Y = 3.87 + 1707.07 X$ ,  $r = 0.9595$ ,  $P<0.001$ ) and reached to 12.9% with the highest concentration. Even though their number in the smears did not alter much, their relative percentage showed about four and a half times increase which might have been due to the fact that most of these cells did not exhibit severe damage as was found in case of plasmatocytes and adipohaemocytes. Besides, all those cells which could not be identified either due to distortion caused during smear making or due to abnormalities caused by the action of insecticides were also included. Consequently, the application of 0.001, 0.002, 0.004 and 0.006% acephate resulted in the appearance of respectively 13.34, 19.92, 23.72, and 45.62% damaged cells after 6 hrs of application exhibiting a positive linear correlation with increasing concentration of acephate as compared to control which showed 6.64% abnormal cells (Table1).

After one day, the density of prohaemocytes progressively and linearly increased with increasing concentrations ( $Y = 17.0529 + 2358.10 X$ ,  $r = 0.8385$ ,  $P<0.001$ )

showing 27.34% ( $t=2.268$ ,  $P>0.05$ ) cells in 0.004% acephate affected smears compared to 11.96% in control. However, by 0.006% acephate there was slight increase in their number compared to 0.004% concentration. The plasmatocytes were highly affected cells. Their population in control and untreated insects was  $43.62\% \pm 3.16$  and  $47.12\%$  respectively. There was a concentration based linear reduction ( $Y = 34.73 - 4977.41 X$ ,  $r = -0.8847$ ,  $P<0.001$ ) in the density of these cells showing a negative correlation coefficient. The highest concentration reduced the population of these cells to 7.26% showing a statistically significant reduction ( $t=4.566$ ,  $P<0.05$ ) compared to control. Similarly, the adipohaemocytes showed an inverse linear relation to the increasing doses of acephate ( $Y = 22.99 - 4252.41 X$ ,  $r = -0.9604$ ,  $P<0.001$ ). Furthermore, following treatment of the highest concentration the adipohaemocytes disappeared from the smears. The population of granulocytes increased to 15.32% and 9.14% following treatment with 0.001 and 0.002% acephate as compared to 8.42% in the control. However, by the higher concentrations (0.004 and 0.006%) the population of granulocytes was reduced to 5.62% and 1.74% compared to control (8.42%). Subsequent to the application of 0.001, 0.002, 0.004 and 0.006% concentrations the relative percentage of oenocytoids was progressively increased linearly ( $Y = 4.897 + 1756.38 X$ ,  $r = 0.9855$ ,  $P<0.001$ ), however, only a few oenocytoids showed damage and most of these cells were recognizable even in the smears of 0.006% acephate affected nymphs. The proportion of damaged and disintegrated cells was enhanced with increase in concentration of insecticide applied showing a positive linear correlation ( $Y = 6.716 + 7002.93 X$ ,  $r = 0.9822$ ,  $P<0.001$ ). The highest concentration caused destruction of 47.56% haemocytes compared to 9.72% in control showing a statistically significant increase ( $t= 4.174$ ,  $P<0.05$ ) (Table2).

Three days following treatment with 0.001 and 0.002% acephate the percentage of prohaemocytes exhibited an increasing trend. However, with 0.004 and 0.006% concentrations there was slight fall in their population compared to lower concentrations. In the smears of control nymphs, plasmatocytes constituted 33.78% of total cell population. Subsequent to the application of 0.001, 0.002, 0.004 and 0.006% concentration, the respective percentage of these haemocytes was 24.58, 14.34, 13.0 and 10.7 showing concentration based linear reduction ( $Y = 28.378 - 3499.14 X$ ,  $r = -0.8694$ ,  $P<0.001$ ) with increasing concentrations of acephate which was

statistically significant at 0.004 and 0.06% concentration ( $t=2.891$  and  $t=3.207$  respectively,  $P<0.05$ ). The adipohaemocytes were most fragile cells and were highly damaged even by lower concentrations. Following 0.002% acephate, the population of adipohaemocytes was reduced to one third. Moreover, by 0.004 and 0.006% acephate the adipohaemocytes were completely damaged. Similarly, the population of granulocytes progressively increased to nearly double by 0.004% concentration. However, following the highest concentration these cells were not found in the smear. Oenocytoids were the least affected cells in the smear. Although, the number of these cells remained constant in the treated insects their relative percentage showed an increase due to disintegration of other types of cells. Following the highest concentration of acephate the population of these cells increased to 17.5% compared to 3.72% in control showing a concentration based linear increase with increase in concentrations ( $Y = 4.256 + 2410.69 X$ ,  $r = 0.9796$ ,  $P<0.001$ ).

There was a consistent increase in the proportion of damaged/unidentified cells in accordance with the increase in concentration of acephate applied ( $Y = 6.849 + 5932.07 X$ ,  $r = 0.9803$ ,  $P<0.001$ ). Thus a maximum of 40.9% ( $t=3.889$ ,  $P<0.05$ ) cells were found damaged/disintegrated with the highest concentration of acephate showing statistically significant increase (Table 3).

After 5 days of the treatment with different concentrations of acephate prohaemocytes showed an increase in their population, highest being in 0.004% acephate treated nymphs. Plasmatocytes were severely affected by 0.004 and 0.006% concentrations comprising only 14.10% and 02.62% of total cells showing statistically significant ( $t=3.655$  and  $t=4.070$ , respectively,  $P<0.05$ ) reduction in their population and having a negative linear correlation ( $Y = 33.034 - 5071.72 X$ ,  $r = -0.9842$ ,  $P<0.001$ ) with increasing concentrations of acephate compared to control (35.82%). Adipohaemocytes were significantly less (19.52%) in 0.002% acephate affected smears ( $t=3.014$ ,  $P<0.05$ ), however, these cells were completely damaged by 0.004 and 0.006% acephate. Similarly, granulocytes, too, were unidentifiable in smears affected with higher concentrations. The population of oenocytoids showed a steady increase with the increase in concentration of acephate displaying a positive linear correlation ( $Y = 4.537 + 2136.38 X$ ,  $r = 0.9813$ ,  $P<0.001$ ). Their relative percentage was almost six and a half times higher in 0.004 and 0.006% acephate treated nymphs. After five days following

application of 0.006% acephate, 47.08% cells were unidentifiable and with 0.004% concentration there were 41.92% cells unidentifiable compared to 6.82% in control thereby showing a statistically significant reduction ( $t=3.192$  and  $t=4.061$ ,  $P<0.05$ ) and exhibiting a positive correlation ( $Y = 3.851 + 7731.21 X$ ,  $r = 0.9673$ ,  $P<0.001$ ) with increasing concentrations. On the other hand, with two lower concentrations, the effect on population was statistically insignificant (Table 4). When the treated nymphs moulted to adult stage, the relative percentage of each type of haemocytes was calculated in males and females separately.

In one-day-old males (Table-5), the prohaemocyte population showed statistically insignificant increase with the application of lower concentrations. However, with 0.004% acephate there were approximately five times more cells in the smear compared to control. Plasmatocyte population inconsistently decreased showing statistically insignificant reduction. Similarly, adipohaemocytes showed insignificant reduction by the application of different concentrations of acephate. Granulocytes and oenocytoids too followed the same trend. Likewise, population of damaged cells showed statistically insignificant change.

In the females emerged from treated nymphs (Table 6) only prohaemocytes showed statistically significant increase by 0.004% acephate ( $t=3.089$ ,  $p<0.05$ ) compared to control. Besides that all the other haemocyte types exhibited inconsistent and statistically insignificant change. Effects of a number of exogenously applied chemicals have been ascertained on differential haemocyte counts. Following exposure (ingestion) of *Periplaneta americana* to chlordane (a chlorinated hydrocarbon) plasmatocytes, granulocytes, spherulocytes and coagulocytes increased in number. (Gupta and Sutherland, 1968). Injection of various doses of B-ecdysone to *Spodoptera litura*, caused an increase in the prohaemocyte and plasmatocyte population and decline in the adipohaemocytes. The effect of B-ecdysone was more progressive after the treated insect moulted to next instar. (Nishi, 1982). George and Ambrose (2004) studied the impact of five organophosphorous insecticides, viz. monocrotophos, dimethoate, methylparathion, quinalphos and endosulfan on the differential haemocyte counts (DHC) of *Rhynocoris kumarii*. All of the insecticides except endosulfan initially reduced both prohaemocytes and plasmatocytes, increased the granular haemocytes, and altered the percentage of cystocytes and oenocytoids. On the contrary, endosulfan initially increased the

prohaemocytes and plasmatocytes, decreased the granular haemocytes. The highest impact on the DHC was caused by methylparathion, monocrotophos, and the least impact by endosulfan.

Haq (2005) studied the toxicity of Nicotinyl insecticides on the haemocytes of *Dysdercus konigi* and reported that the percentage of plasmatocytes, granulocytes and prohaemocytes decreased from the normal and that oenocytoides and spherulocytes increased after application of acetamiprid 20% SL, whereas application of Imidacloprid 25 WP caused a reduction in plasmatocytes, granulocytes, oenocytoides and spherulocytes and increase in prohaemocyte population. Application of varied concentrations of Neem gold on *Spodoptera litura* 24 to 72 h after treatment resulted in a decrease in PRs, PLs, SPs and increase in GRs and OEs, which was more pronounced after 48 to 72 h treatment at a concentration of 1500 ppm of Neem gold (Sharma et al 2003).

Berger et al (2003) reported slight increase in relative number of prohaemocytes and slight decrease in proportion of plasmatocytes and granulocytes in *Periplaneta americana* after subchronic treatment with genistein, a phytoestrogen isoflavanoid, whereas the same chemical induced significant increase in the prohaemocyte proportion following single or subchronic treatment. Different types of haemocytes respond differently to stress conditions as evident by observations on different insect species treated with different compounds having different chemical composition. A more elaborate and thorough study is required including more insect species belonging to diverse insect orders before drawing any definite conclusion or inference regarding the response of various haemocytes to insecticidal stress.

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**Table 1: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Acephat on 1-2 day old 5<sup>th</sup> instar nymphs of *Dysdercus cingulatus***

Concentrations (%) 1 $\mu$ l / nymph	Prohaemocytes % $\pm$ S. E.	Plasmatocytes % $\pm$ S. E.	Adipohaemocytes % $\pm$ S. E.	Granulocytes % $\pm$ S. E.	Oenocytoids % $\pm$ S. E.	Disintegrated Cells % $\pm$ S. E.
Untreated	09.08 $\pm$ 0.85	53.02 $\pm$ 5.15	16.58 $\pm$ 3.23	08.50 $\pm$ 1.15	04.00 $\pm$ 1.47	08.92 $\pm$ 2.55
Solvent Treated	09.26 $\pm$ 1.16	53.20 $\pm$ 4.32	17.06 $\pm$ 3.51	10.44 $\pm$ 3.07	03.16 $\pm$ 1.19	06.64 $\pm$ 1.69
0.001	18.18 $\pm$ 2.06	36.78 $\pm$ 4.39	15.84 $\pm$ 1.47	09.92 $\pm$ 0.81	05.94 $\pm$ 1.19	13.34 $\pm$ 3.00
0.002	18.80 $\pm$ 2.08	34.96 $\pm$ 3.56	11.32 $\pm$ 1.58	08.68 $\pm$ 0.56	06.96 $\pm$ 0.97	19.92 $\pm$ 2.23
0.004	23.46 $\pm$ 2.60	22.58 $\pm$ 3.00	09.72 $\pm$ 2.30	07.72 $\pm$ 1.61	12.60 $\pm$ 1.70	23.72 $\pm$ 2.39
0.006	27.16 $\pm$ 2.26	08.86 $\pm$ 1.78	00.00	05.36 $\pm$ 1.46	12.90 $\pm$ 0.60	45.62 $\pm$ 2.37

**Table 2: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Acephate on 1-2 day old 5<sup>th</sup> instar nymphs of *Dysdercus cingulatus***

Concentrations (%) 1 $\mu$ l / nymph	Prohaemocytes % $\pm$ S. E.	Plasmatocytes % $\pm$ S. E.	Adipohaemocytes % $\pm$ S. E.	Granulocytes % $\pm$ S. E.	Oenocytoids % $\pm$ S. E.	Disintegrated Cells % $\pm$ S. E.
Untreated	11.30 $\pm$ 2.19	47.12 $\pm$ 5.16	23.62 $\pm$ 4.08	06.56 $\pm$ 1.14	03.60 $\pm$ 1.30	07.88 $\pm$ 1.94
Solvent Treated	11.96 $\pm$ 2.15	43.62 $\pm$ 2.48	22.14 $\pm$ 3.34	8.42 $\pm$ 0.87	04.12 $\pm$ 0.99	09.72 $\pm$ 1.64
0.001	22.02 $\pm$ 2.05	23.30 $\pm$ 1.31	21.14 $\pm$ 2.42	15.32 $\pm$ 2.23	07.30 $\pm$ 1.04	10.76 $\pm$ 1.51
0.002	25.72 $\pm$ 2.48	10.08 $\pm$ 2.86	15.10 $\pm$ 3.28	12.84 $\pm$ 1.56	09.14 $\pm$ 0.87	17.98 $\pm$ 1.92
0.004	27.34 $\pm$ 4.54	15.70 $\pm$ 2.16	01.28 $\pm$ 0.79	05.62 $\pm$ 1.49	11.18 $\pm$ 0.66	38.60 $\pm$ 2.81
0.006	28.88 $\pm$ 1.89	07.26 $\pm$ 1.42	00.00	01.74 $\pm$ 0.37	15.58 $\pm$ 2.59	47.56 $\pm$ 3.21

**Table 3: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Acephate on 1-2 day old 5<sup>th</sup> instar nymphs of *Dysdercus cingulatus***

Concentrations (%) 1 $\mu$ l / nymph	Prohaemocytes % $\pm$ S. E.	Plasmatocytes % $\pm$ S. E.	Adipohaemocytes % $\pm$ S. E.	Granulocytes % $\pm$ S. E.	Oenocytoids % $\pm$ S. E.	Disintegrated Cells % $\pm$ S. E.
Untreated	15.48 $\pm$ 1.55	32.78 $\pm$ 2.74	31.12 $\pm$ 4.15	10.84 $\pm$ 1.78	01.98 $\pm$ 1.07	07.68 $\pm$ 1.34
Solvent Treated	17.00 $\pm$ 2.55	33.78 $\pm$ 2.74	30.34 $\pm$ 2.74	06.64 $\pm$ 1.25	03.72 $\pm$ 0.94	08.78 $\pm$ 1.27
0.001	24.26 $\pm$ 2.05	24.58 $\pm$ 1.39	20.12 $\pm$ 2.03	14.08 $\pm$ 1.47	05.90 $\pm$ 1.27	10.92 $\pm$ 2.25
0.002	34.56 $\pm$ 1.95	14.34 $\pm$ 3.42	10.76 $\pm$ 1.16	14.18 $\pm$ 1.89	10.08 $\pm$ 1.15	16.10 $\pm$ 1.52
0.004	18.74 $\pm$ 4.36	13.00 $\pm$ 2.81	00.00	18.18 $\pm$ 1.22	15.42 $\pm$ 2.00	34.66 $\pm$ 2.78
0.006	30.84 $\pm$ 2.85	10.70 $\pm$ 2.27	00.00	00.00	17.50 $\pm$ 1.87	40.90 $\pm$ 3.48

**Table 4: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Acephate on 1-2 day old 5<sup>th</sup> instar nymphs of *Dysdercus cingulatus***

Concentrations (%) 1 $\mu$ l / nymph	Prohaemocytes % $\pm$ S. E.	Plasmatocytes % $\pm$ S. E.	Adipoaemocytes % $\pm$ S. E.	Granulocytes % $\pm$ S. E.	Oenocytoids % $\pm$ S. E.	Disintegrated Cells % $\pm$ S. E.
Untreated	06.30 $\pm$ 1.97	32.21 $\pm$ 4.63	39.18 $\pm$ 2.60	13.44 $\pm$ 1.87	04.26 $\pm$ 0.80	05.34 $\pm$ 0.99
Solvent Treated	06.08 $\pm$ 1.17	35.82 $\pm$ 2.61	31.64 $\pm$ 2.39	15.92 $\pm$ 1.75	03.78 $\pm$ 0.63	06.82 $\pm$ 1.57
0.001	17.06 $\pm$ 3.30	25.16 $\pm$ 2.77	23.60 $\pm$ 5.34	12.68 $\pm$ 1.73	07.44 $\pm$ 1.89	07.30 $\pm$ 1.30
0.002	22.36 $\pm$ 4.54	21.54 $\pm$ 1.19	19.52 $\pm$ 0.59	11.66 $\pm$ 1.34	08.30 $\pm$ 1.11	16.64 $\pm$ 2.22
0.004	29.58 $\pm$ 3.13	14.10 $\pm$ 1.62	00.00	00.00	14.46 $\pm$ 3.70	41.92 $\pm$ 3.19
0.006	25.64 $\pm$ 2.45	02.62 $\pm$ 1.87	00.00	00.00	16.48 $\pm$ 2.81	47.08 $\pm$ 7.27

**Table 5: Differential Haemocyte Counts determined in one day old adult male following the topical application of various concentrations of Acephate on 1-2 day old 5<sup>th</sup> instar nymphs of *Dysdercus cingulatus***

Concentrations (%) 1 $\mu$ l / nymph	Prohaemocytes % $\pm$ S. E.	Plasmatocytes % $\pm$ S. E.	Adipoaemocytes % $\pm$ S. E.	Granulocytes % $\pm$ S. E.	Oenocytoids % $\pm$ S. E.	Disintegrated Cells % $\pm$ S. E.
Untreated	02.48 $\pm$ 1.13	39.66 $\pm$ 5.25	45.30 $\pm$ 4.84	06.76 $\pm$ 1.53	02.20 $\pm$ 0.62	03.56 $\pm$ 0.71
Solvent Treated	2.86 $\pm$ 0.80	37.32 $\pm$ 4.23	44.34 $\pm$ 5.02	07.20 $\pm$ 1.91	01.36 $\pm$ 0.49	05.92 $\pm$ 2.39
0.001	03.20 $\pm$ 0.45	31.92 $\pm$ 2.84	42.74 $\pm$ 4.02	06.78 $\pm$ 1.63	05.44 $\pm$ 1.29	09.92 $\pm$ 2.51
0.002	07.86 $\pm$ 1.65	28.58 $\pm$ 3.71	33.06 $\pm$ 4.74	09.98 $\pm$ 1.60	04.74 $\pm$ 0.94	13.80 $\pm$ 1.61
0.004	15.10 $\pm$ 2.81	31.84 $\pm$ 5.82	33.84 $\pm$ 5.18	07.26 $\pm$ 2.26	01.58 $\pm$ 0.77	10.44 $\pm$ 1.10
0.006	00.00	00.00	00.00	00.00	00.00	00.00

**Table 6: Differential Haemocyte Counts determined in one day old adult females following the topical application of various concentrations of Acephate on 1-2 day old 5<sup>th</sup> instar nymphs of *Dysdercus cingulatus***

Concentrations (%) 1 $\mu$ l / nymph	Prohaemocytes % $\pm$ S. E.	Plasmatocytes % $\pm$ S. E.	Adipoaemocytes % $\pm$ S. E.	Granulocytes % $\pm$ S. E.	Oenocytoids % $\pm$ S. E.	Disintegrated Cells % $\pm$ S. E.
Untreated	05.78 $\pm$ 1.77	50.96 $\pm$ 2.11	28.54 $\pm$ 3.41	06.30 $\pm$ 1.74	01.14 $\pm$ 0.47	07.36 $\pm$ 1.70
Solvent Treated	05.05 $\pm$ 1.72	50.52 $\pm$ 5.93	28.18 $\pm$ 3.78	06.64 $\pm$ 1.61	01.82 $\pm$ 0.64	05.74 $\pm$ 1.31
0.001	07.88 $\pm$ 0.66	52.52 $\pm$ 2.09	22.26 $\pm$ 3.00	07.52 $\pm$ 1.24	01.86 $\pm$ 0.26	07.94 $\pm$ 1.28
0.002	13.98 $\pm$ 7.56	26.74 $\pm$ 1.98	25.20 $\pm$ 2.89	09.68 $\pm$ 0.72	02.62 $\pm$ 0.80	10.20 $\pm$ 1.49
0.004	18.52 $\pm$ 1.44	32.40 $\pm$ 1.68	23.40 $\pm$ 1.14	11.06 $\pm$ 0.60	02.98 $\pm$ 0.75	11.80 $\pm$ 0.89
0.006	00.00	00.00	00.00	00.00	00.00	00.00