CHANGES IN HAEMOCYTO COUNTS FOLLOWING TOPICAL APPLICATION OF $\beta$-ECYDSONE AND MAKISTERONE A ON 5th INSTAR NYMPHS OF Dysdercus cingulates FABR. (HEMIPTERA, PYRRHOCORIDAE)

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Received 2 7, 1994.

Topical application of sub-lethal doses (0.5, 1, 2, 4 and 6 $\mu$g/nymph) of $\beta$-ecdyson and Makisterone A (a phytoecdysone) on 5th instar nymphs (24 h old) subsequently caused dose-based drop in total haemocyte counts (THC). After 48 hours THC was only 25.5 $\%$ and 31.4 $\%$ with respect to 6 $\mu$g $\beta$-ecdyson and Makisterone A. But later in adults correspondingly there was appreciable increase in THC. However, in F1 generation there was further reduction. The differential haemocyte counts were made on the basis of cells showing respective characteristics. Thus in affected 5th instar nymphs (72 h old), effect was also dose-based. By the strongest dose of $\beta$-ecdyson, adipohemocytes were completely destroyed and plasmocytes were only 70 $\%$ against control. Therefore, the percentage of prohaemocytes and oenocytoids which were less affected, were found highly increased. Similar nature of trend was observed with respect to Makisterone A. In emerged adults also there was selective damage on different haemocytes like that of nymphal stage. Although their distinguishing features were lost by the strongest dose. Both the hormones are toxic to the haemocytes but $\beta$-ecdyson proved more fatal than Makisterone A.

Dysdercus cingulates, $\beta$-ecdysone, haemocytes, Makisterone A, total haemocyte counts (THC), differential haemocyte counts (DHC).

AHMAD, A., Department of Zoology, Aligarh Muslim University, Aligarh - 202001 (U.P.), India. - Promjera broja hemocita nakon površinske aplikacije $\beta$-ekdisona i makisterona A na nimfama 5-go stadija Dysdercus cingulates Fab. (Hemiptera, Pyrrhocoridae). - Entomol. Croat., 1995. Vol. 1: 41 - 48. - Površinska aplikacija subletalnih doza (0,5, 1, 2, 4 i 6 $\mu$g/nimfa) $\beta$-ekdisona i makisterona A (fitoksidon) na nimфama 5-go stadia, stare 24 sati, uzrokuje nizamudno, ovisno o dozi, pad ukupnog broja hemocita (THC). Nakon 48 sati THC je bio samo 25,5 $\%$ i 31,4 $\%$ kod primjene 6 $\mu$g $\beta$-ekdisona i makisterona A. Ali kasnije kod imaga desilo je do odgovarajućeg značajnog povećanja THC. Međutim, kod F1 generacije dolazi do daljnjeg snimanja. Diferencijalni broj hemocita izmjerno je na temelju stanaca koje su pokazale odgovarajuće karakteristike. Tako je kod napadnutih nimfa 5-go stadia, starih 72 sati, učinak također bio ovisan o dozi. Kod najjačih doza $\beta$-ekdisona, adipohemociti su bili potpuno uništeni a plazmatociti je bilo samo 70 $\%$ od kontrole. Stoga je postojao prohemocit i oenocit, koji su bili manje napadnuti, bio znatno povećan. Sličan je trend zapažen kod primjene makisterona A. Kod izazivanih imaga također je bilo različito oštećenje raznih vrsta hemocita kao i kod nimfa, primornja se različitost pojave gubi kod najjačih doza. Oba su hormona toksična za hemocite ali se $\beta$-ekdison pokazao smrtonosnijim od makisterona A.

Dysdercus cingulates, $\beta$-ekdison, hemociti, makisteron A, ukupni broj hemocita (THC), diferencijalni broj hemocita (DHC).
Introduction

In the recent years, the effect of application of exogenous ecdysones (α- and β-ecdysones) and their synthetic analogues as well as ecdysones of plant origin - phytocdysones - have been studied on a variety of insects with regard to assess their effects on growth and reproduction. But the effect of these hormones on haemocytes is little known. Nishi (1982) and the author (1993) observed the decreasing trend of total haemocyte counts (THC) in Spodoptera litura. Similar observation was made by Khan et al. (1984) in the nymphs and adults of Heteroglyphus nigrorepletus. But Rao et al. (1984) found significant increase in total haemocyte counts in S. litura by the injection of β-ecdysone.

In the present work on cotton stainer bug Dysdercus cingulatus, changes in THC and differential haemocyte count (DHC) of 5th instar nymphs and adults (3 days old) have been studied following the topical application of 0.5, 1, 2, 4 and 6 µg of β-ecdysone and Makisterone A on individual 5th instar nymphs (24 h old).

Material and Methods

Stock culture of D. cingulatus was maintained at 30 ± 1°C and 70-80% R.H. Soaked healthy cotton seeds were given daily as food. Newly moulted 5th instar nymphs were sorted out for experiment.

Effect of these hormones was studied by applying (topically) its different doses i.e. 0.5, 1, 2, 4 and 6 µg in (1 µl) acetone solution on the individuals of 5th instar nymphs (24 h old). For control, 5th instar untreated nymphs and nymphs treated with acetone only of the similar stage and age were kept in separate rearing jars at the same temperature and humidity.

1. Total haemocyte counts (THC): Fresh blood of individual nymph was drawn in the pipette used for counting human leucocytes. This blood was diluted with a modified diluting fluid (1% glacial acetic acid with a pinch of gentian violet to stain the haemocytes). Total haemocytes/mm³ were counted with the help of improved Neubauer counting chamber described by Damady and Davenny (1963) and Johansen (1969). The total number of haemocytes/mm³ was calculated by a formula X/4 x 10 x 20, where X = total number of haemocytes in 4 corner squares of the chamber.

4 = Number of corner squares
10 = Reverse of depth of the counting chamber of haemocytometer (1/10 mm)
20 = Dilution of the haemolymph.

2. Differential haemocyte counts (DHC): The percentage of different types of haemocytes was calculated on the basis of the number of each type of haemocyte in permanent and stained preparations. In each preparation three areas, each of 1 cm² was selected for counting. The criterion for this selection was the uniform distribution of haemocytes and each type of cell at a particular stage was calculated by counting ten separate preparations from the respective number of insects treated or untreated. Ten insects were used for DHC of each dose and one slide was prepared from one insect.

Results

Total haemocyte counts following topical application of different doses of β-ecdysone on 5th instar nymphs (24 h old).

1. In F generation: In the affected 5th instar nymphs (72 h old) the damage of cells was dose-based and so the THC was dropped. But the reduction of cells was comparatively less in insects treated with Makisterone A than that of β-ecdysone. THC was only 25.5% as compared to control by β-ecdysone. Whereas 31.4% by Makisterone A. Likewise in the emerged adults, THC was 65.6% in males and 65% in females by β-ecdysone and 62.4% in males and 86.2% in females by Makisterone A (Figs. 1 & 2).

Fig. 1 & 2. THC of 5th instar nymphs and adults (3 days old) of Dysdercus cingulatus in F generation following topical application of different doses of β-ecdysone and Makisterone A respectively on 5th instar nymphs (24 h old).
2. In F1 generation: When the females emerged from the treated nymphs (by β-ecdysone) were mated separately with males of the control stock of the same age, their eggs hatched. Then in the 4th instar nymphs of F1 generation, the fall in THC of these nymphs was dose-based and maximum decrease was found with respect to 6 µg dose in both 4th and 5th instars (57 % and 76.4 % respectively). Whereas in the affected insects of Makisterone A, THC was 48.2 % in 4th instar nymphs, 26 % in 5th instar nymphs. The adults of this generation also had reduced THC which were 56.6 % in males and 50 % in females (by β-ecdysone) and 62 % in males and 60 % in females (by Makisterone A) (Figs. 3 & 4).

**Differential haemocyte counts (DHC) of *D. cingulatus* nymphs (5th instars and adults, 3 days old) following topical application of different doses of β-ecdysone and Makisterone A on 5th instar nymphs (24 h old)**

When treated nymphs became 3 days old, maximum effect on THC was due to 6 µg dose of each hormone. The percentage of prohaemocytes (PRs) increased to 2.7 times as compared to that of control. The percentage of plasmocytes (PLs) decreased to 70 % and that of oenocytes (OE) increased to 4.2 times as compared to control. Whereas adipohaemocytes (ADs) were completely destroyed even by the application of the lowest dose (β-ecdysone). Further, by Makisterone A PRs increased to 1.44 times, PLs to 1.3 times and the percentage of OEs was raised to 2.7 times as that of control.

In the affected adults from the treated nymphs by 4 and 6 µg β-ecdysone, all types of cells lost their distinguishing features and could not be identified. Therefore, counting was possible only in the haemolymph of adults affected by 2 µg dose. The percentage of PRs dropped in the males affected by 0.5 and 1 µg β-ecdysone but slightly increased with respect to 2 µg dose. Whereas the percentage of PLs increased by 0.5 and 1 µg doses but decreased by 2 µg dose. The percentage of OEs enormously increased and became 18 times of the control. As described earlier ADs were completely damaged in the smear. OEs were again the most resistant cells. Similar pattern of DHC was seen by the affected females.

Similarly by 2 µg Makisterone, the percentage of PRs increased to 1.6 times in males and 1.64 times in females. The percentage of PLs decreased by 55 % in males and 50 % in females. The percentage of OEs increased to 31 times in males and 22 times in females. There was no trace of ADs in the smears (Figs. 5 & 6).

**Discussion**

The present data reveals that exogenous application of hormones caused damage to the haemoocytes, which resulted in great reduction of THC. There is dose-based drop in THC value and it is more pronounced by the application of β-ecdysone as compared to Makisterone A. The residual effects of these hormones were also noted in 4th and 5th instar nymphs and adults of the next generation, which almost showed the same trend. Similar results were recorded by N i s h i (1982) and the author (1993) in *Spodoptera littoralis* and in *Heterophasis nigrorepleta* by K h a n et al. (1984). In contrast to this R a o et al. (1984) in *S. littoralis* noticed the significant increase in the cell numbers when injected β-ecdysone. G u c c i and S t e r l i n g (1968) in *Periplaneta americana* also reached to the same conclusion when treated with an insecticide chlordane.

As far as DHC is concerned, percentage of PRs apparently increased as compared to control by the strongest dose of both the hormones. OEs also increased enormously as compared to control. Whereas percentage of PLs decreased and that ADs dropped and became nil. PRs and OEs showed apparent increase in their percentage even by the effect of the strongest dose because of higher rate of destruction of other types of cells in the smear. In emerged adults DHC were possible only in the haemolymph affected by doses of 0.5 to 2 µg because higher doses destroy the distinguishing features of all types of haemoocytes. The cells which were identifiable after the treatment by lower doses showed the same type of damage.
Fig. 5. DHC of 5th instar nymphs and adults (3 days old) of *Dysdercus cingulatus* following topical application of different doses of β-ecdysone on 5th instar nymphs (24 h old).

The effect of DHC by exogenous application of hormones is recorded by Nishii (1982) and the author (1993) in *S. itiera* which is similar to the present data.

In the present experiment it is concluded that ecdysones, exogenously applied cause pathogenicity in the haemocytes. By increasing the titre of ecdysone, it may be harmful to the growth in general. Therefore the application of lethal doses of these hormones can be used in controlling insect pests of agricultural crops, if economically feasible.

Fig. 6. DHC of 5th instar nymphs and adults (3 days old) of *Dysdercus cingulatus* following topical application of different doses of Makisterone A on 5th instar nymphs (24 h old).

Acknowledgements

Thanks are due to Indian Council of Agricultural Research for the grant of financial assistance.
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