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The effects of juvenile hormone analogue ZR-512 on egg hatching and embryonic development of ***Melanogryllus desertus*** Pall. (Orthoptera: Gryllidae)

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Summary

The effects of Juvenile hormone analogue ZR-512 on egg hatching and embryonic development of ***Melanogryllus desertus*** were investigated. Different doses of ZR-512 in 1 µl acetone were topically applied on 7-days-old eggs in early blastokinesis.

The effective dose was found 30.83 ppm for inhibiting egg hatching at hundred percent while the concentration which has a 50% inhibitory effect was 5.957 ppm Uneffective concentration on egg hatching was 1.384 ppm.

Different concentrations of ZR-512 caused some abnormalities in embryonic structures such as in development of mouth parts, antennae, cerci, legs, spines and body pigmentation. There were some differences on the amount of utilized vitellus. The results indicated that ZR-512 was an effective hormone analogue in the egg hatching and embryonic development of ***M. desertus***.

Key words: Juvenile hormon analogue ZR-512, egg hatching, embryonic development, ***Melanogryllus desertus***, Orthoptera

Anahtar sözcükler: Juvenil hormon analoğu ZR-512, yumurta açılması, embriyonik gelişim, ***Melanogryllus desertus***, Orthoptera

Introduction

The control against to harmful insects, the existing insectisides were insufficient for some reasons. So, this leaded insect physiologs to search for new methods (Geldiay, 1971). The opinion that insect hormones named as insect growth regulators were the compounds which constituted the third group of

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pestisides and had no harmful effect on human, nature and environment, gained importance for long years (DeWilde, 1964, 1971; Williams, 1967; Ellis et al., 1970; Schneiderman, 1971, 1972; Staal, 1972, 1974; Kısmalı & Schooneveld, 1979; Sehnal, 1983; Hicks & Gordon, 1992; 1994; Sigh, 1994).

Insect neuroendocrine system is composed of brain neurosecretory cells producing neurohormones, neurohaemal regions storing and releasing neurohormones and endocrine glands like corpora allata and prothorasic glands which are connected with neurosecrotory cells by nerves. The importance of the insect neuroendocrine system on growth, maturation, metamorphosis, reproduction and diapause was understood and its active role was shown in the control of these events by the experimental works supported with histological and ultrastructural researches on various kinds of insects. In the end of the studies on the Juvenil hormone which is released from corpora allata, new compounds with the same effect were found. These are synthetic Juvenile hormone (SJH) or Juvenile hormone analogues (JHA). The idea of using these compounds as insectisides, came out first from Williams (1960). Schmialek (1961) first found the Farnesal from ***Tenebrio molitor*** L. (Coleoptera: Tenebrionidae) showed Juvenile hormone activity. The chemical structure of the hormone was determined by Röller et al. (1967, 1968). After this study, hundreds of compounds which showed Juvenile hormone activity were found (Slama et al., 1974; Novak, 1975). The effects of synthetic Juvenile hormone and Juvenile hormone analogues on insect embryogenesis (Slama & Williams, 1966; Riddiford & Williams, 1967; Novak, 1969; Matolin, 1970; Staal, 1972; Hunt & Shappirio, 1973; Rohdendorf & Sehnal, 1973; Troisi & Riddiford, 1974; Smith & Arking, 1975; Geldiay et al., 1978, 1981; Kısmalı & Erkin, 1984; Hicks & Gordon, 1992, 1994; Madanlar & Kısmalı, 1994; Singh, 1994) and on ovary development (Slama & Williams, 1966; Riddiford, 1972; Rohdendorf & Sehnal, 1973; Singh, 1994; Schneider et al., 1995) were researched by many scientists on various species of insects. Kısmalı (1979, 1983), found that juvenile hormone analogues could be useful for the control of aphids. Nemec (1995), Hattingh & Tate (1996) suggested that insect hormone analogues are very useful compounds in harmful insect control. Field studies on insects are being made with the active compounds that gave positive results.

It is well known phenomenon that some changes occuring in hormone level in hemolymph causes growth and reproduction abnormalities. The idea for using the insect hormones to control harmful insects is depended on this phenomenon. Based on these informations, the aim of this study is to determine the effective limits of the concentrations of ZR-512 to control of the black grasshopper, ***Melanogryllus desertus*** Pall. (Orthoptera: Gryllidae) which has significant harm for culture plants in our country (Gümüşsuyu, 1973). Data obtained from this type of studies will provide a base for applied researches.

Material and Method

Maintaining *Melanogryllus desertus* culture

The insects were reared in jars at 28-30°C, 45%-50% the relative humidity

and in an 9-hr light, 15-hr dark photoperiod in culture room. For nutrition, dry chicken fodder and fresh lettuce leaf were given. The water need of insects was supplied by glass tubes filled with water and closed slightly by cotton-wool. Dark coloured, twisted paper pieces were put into culture barrels where adult insects be able to hide in. Adult mature insects selected at the same age were transfered into new culture vessels for laying eggs where petri dishes muffled with constantly moistuired cotton-wool were placed in. The eggs were collected from the cotton wool packages daily and tucked in to new cotton-wool in groups of 50-100 that placed in plastic containers with covers in order to continue their development. Careful attention was paid to keep the egg packages moisty in sufficient level, because the eggs do not develop well in dry conditions and spoil in too much water. Under these conditions, the eggs were hatched in forthnight time (Akyurtlaklı, 1983).

The preparation and application of the hormone

Juvenile Hormone Analogue ZR-512 was supplied from Prof. Dr. Semahat Geldiay with gratitude that was given her by Zoecon as a gift, and consisted of ethyl 3,7,11-trimethyl-2-4=dodecadienoate. Hormone was just weighed and was dissolved in acetone of appropriate volume for the concentrations and it was used immediately after preparation.

Hormone concentrations from a series of 10, 100, 1000, 10 000 ppm of ZR-512 were applied as 1 µl/egg at the initial experiments. Later concentrations 1-100 ppm in progressive order of 1, 2, 4, 6, 8, 10, 20, 40, 80, 100 were tested. The eggs, untreated and show-treated with aceton were also emulated. The experiments had 6 replicates. More than 100 eggs were scoped for 1-40 ppm concantration ranges, but, more than 50 were considered for 40-100 ppm.

Choosing of eggs, the way of application and the evaluation methods of diagnosis

Applications were done on 7 days old eggs which were in early blasto-kinesis stage that their eye pigments had not developed yet. These eggs were chosen under stereomicroscope to provide an uniform experimental material and they were plaud on the parafilm membrane with a drop of water. After removing the excess water from the eggs, 1 µl acetone containing ZR-512 was dropped with Hamilton microsyringe, covering the whole egg. As soon as acetone evaporated the eggs were put into petri dishes on moist cotton-wool and filter paper. They, then were incubated to develop at constant temperature (28°C).

The eggs were controlled every day and separeted into groups according to their grade of vitellus utilization. 7 days after the onset of the experiments, untreated and acetone treated control eggs started to hatch. The number of achieved nymphs were counted and the rate of hatching were calculated. The photographs were taken under stereomicroscope.

The statistical calculations of experimental values obtained from the end of the applications were compared with the Milimetric and Logarythmic Method

(Düzgüneş, 1972). The hormone concentrations that blocked the hatching of eggs in 100 %, 50 % and uneffective levels were determined.

Results and Discussion

The effects of ZR-512 on egg hatching

The embryonic development of acetone control eggs was similar to normal controls and hatching rate was 100 %. In 100 ppm, 1000 pmm, 10 000 ppm concentrations of ZR-512, egg hatching was stopped 100 %. At the drawn graphic based on these data, the hatching rate was rapidly decreased in less than 10 ppm concentrations. The concentration which stopped egg hatching in the rate of 50 % was approximately 6 ppm (Figure 1).

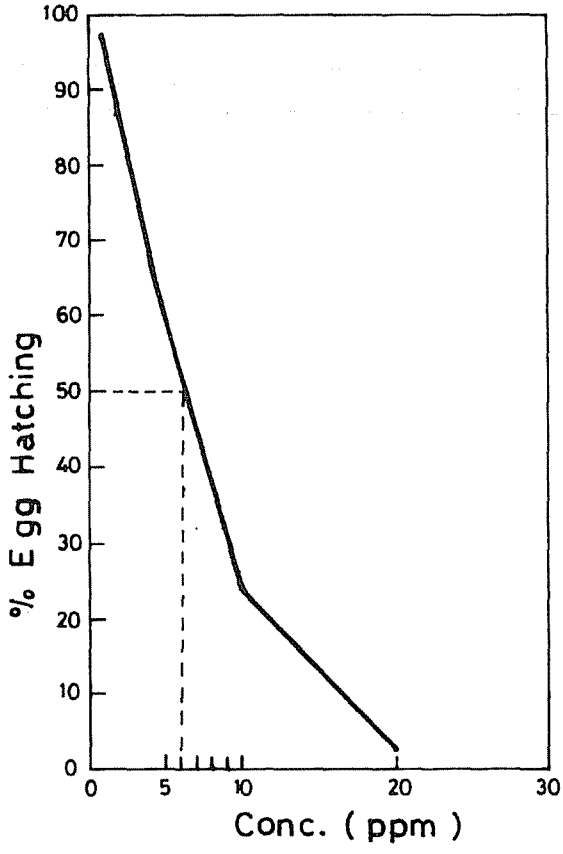

Figure 1. The effects of ZR-512 on hatching of topically treated eggs of ***Melanogryllus desertus***.

Beside the normal and acetone controls, the rates of egg hatching percentages in the applied concentrations of 1-100 ppm can be seen in Table 1. At the end of the experiments, the concentration which stoped 100 % of egg hatching was 40 ppm The hatching rate of 8 ppm concentration is 41.83 and

angle value is 40.28. 6 ppm concentrations' hatching rate is 53.65 and angle value is 47.06.

Table 1. Percentage of egg hatching, means and their angles values of ZR-512 application on ***Melanogryllus desertus***

Conc. (ppm)	The rates of egg hatching (%)							
	E1	E2	E3	E4	E5	E6	Mean	Angle value
1	97	100	90	100	100	100	97.83	81.47
2	73.1	90	85	96	93.3	86.6	87.43	69.21
4	60	70	65	72	66.6	66.6	66.7	54.76
6	55	60	55	52	53.3	46.6	53.65	47.06
8	41.6	40	35	48	46.6	40	41.83	40.28
10	24.5	25	25	28	26.6	20	24.85	29.87
20	9.5	5	0	0	0	0	2.4	8.91
40	0	0	0	-	-	-	-	
60	0	0	0	-	-	-	-	
80	0	0	0	-	-	-	-	
100	0	0	0	-	-	-	-	
Aseton	100	100	100	100	100	100	100	90
Normal	100	100	100	100	100	100	100	90

The values obtained from experiments, "F" test and regression calculations show that there is a high correlation between hormone concentrations and egg hatching rate of angle value. The equation of regression curve Y=83.06 - 49.06 log x, was found as angle value.

According to the results of this equation, Juvenile hormone analogue ZR-512's concentration which stopped 100 % egg hatching (angle value 90) was calculated as 30.83 ppm 50 % stopping concentration (angle value 45) is 5.957 ppm, (Figure 2) 0 % uneffective concentration (angle value 0) is 1.384 ppm.

While examining the effects of Juvenile hormone in embryonic development, the egg hatching was taken as a determinant measure (Slama et al., 1974). The µl value of Juvenile hormone amount which is accepted as standart unit, causes to stop 50 % of egg hatching and named as ID_{50} ovic = inhibation dose-50 ovicidal. When 1 µl of concentration of 1 ppm ZR-515 Altosid in acetone applied on each egg of ***M. desertus*** (Geldiay et al., 1981), 0.0010 µg/l egg active ingredient was given. The active ingredient in the value of ID_{50} ovicidal became 0.004225 µg/l. In this study, the ID_{50} ovic. value for ZR-512 was found as 0.00595 µg/l egg. This value present in ***M. desertus*** is approximately the same with the compounds numbered III and VII which were treated on ***Thermobia domestica*** Packard (Thysanura: Lepismatidae) (Rohdendorf & Sehnal, 1973).

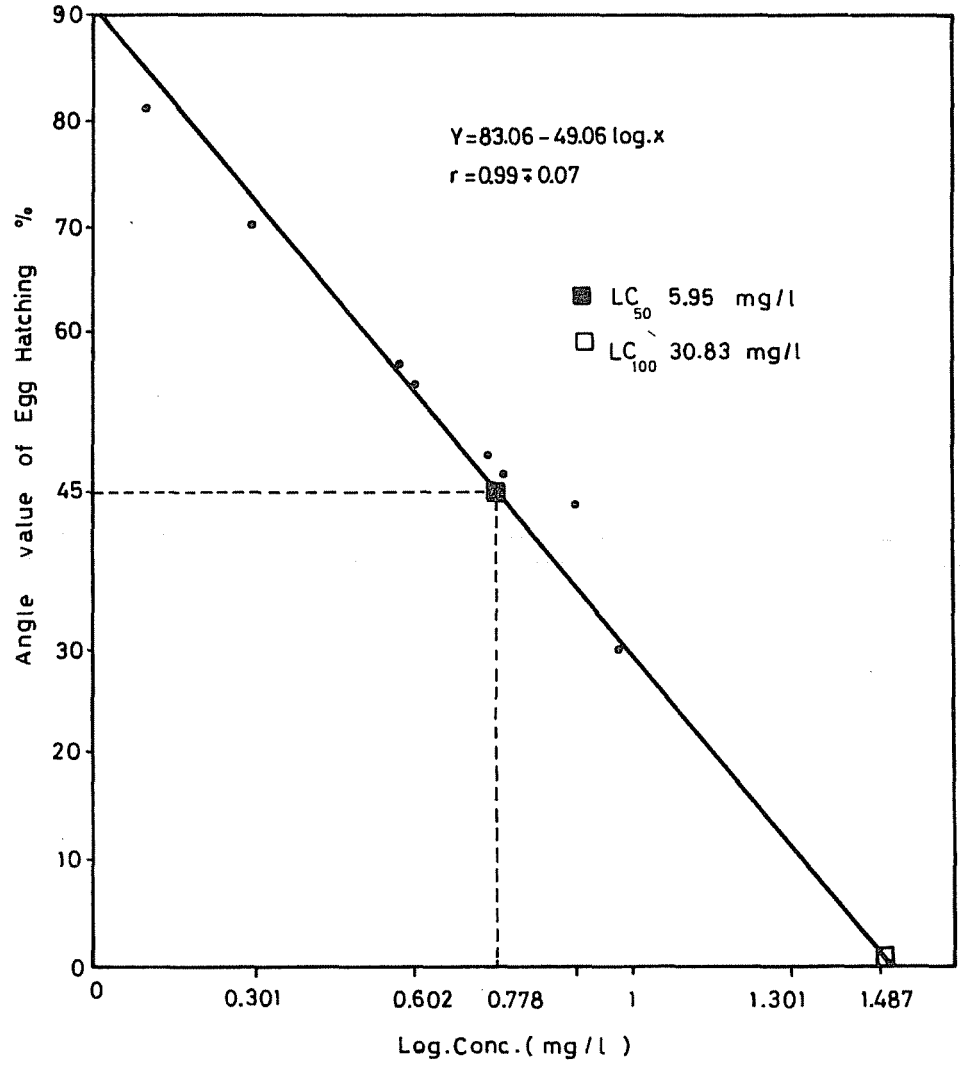

Figure 2. The relation between logarithmic values of applied concentrations of ZR-512 and rate of ***Melanogryllus desertus*** eggs hatching angle values.

Under standart development conditions, the eggs of ***M. desertus*** of the normal and acetone control, hatch in 14 days (Akyurtlaklı, 1983). The eggs were completed their development and hatched in untreated and in acetone controls after seven days of application. The time of hatching in control and in experimental eggs were the same and no relation was found between the used concentrations and time of egg hatching. This result is harmonious with the result which was obtained from the senthetic Juvenile hormone applied eggs of ***Lygaeus kalmii*** L. (Heteroptera: Lygaeidae) however, 2-3 days of delays were occured in the hormone applied eggs of ***T. domestica*** (Rohdendorf & Sehnal, 1973). Hatching percentage was 47 % in control and hormone applied eggs (0.25 and

2.5 µg) of ***L. kalmii*** (Hunt & Shappirio, 1973). Amounts as 0.16, 1.6, 16.0 and 160 (g/mg 25 eggs (practically the amount per one egg should be 0.0016 -1.6) did not cause any embryonic death in ***Drosophila melanogaster*** Meig. (Diptera: Drosophilidae) eggs. When 1.6 and 160 (mg/µl doses were applied, larvae were died after 2 hours from egg hatching during the first instar (Smith & Arking, 1975).

The most sensitive developmental stage was embriogenesis among the examined stages of development of ***Choristoneura fumiferana*** Clemens (Lepidoptera: Tortricidae) during the investigation of Juvenile hormone analogue named fenoxycarb and egg hatching was prevented by 1.5 - 2.5 µl applied doses for each egg (Hicks & Gordon, 1992). It was reported that, resistancy of this compound in the field was relatively high, so it contains a potential for control of particular stages, selectively. 10 ppm doses of ZR-512, -515, -777 were effected reproduction of ***Myzus persicae*** Sulz. (Homoptera: Aphididae) by inhibition (Kısmalı, 1979, 1983). But there was no effect on predatory mite ***Phytoseiulus persimilis*** Athias-Henriot (Acarina: Phytosiidae) (0-18hr old) eggs (Madanlar & Kısmalı, 1994). They have reported that Juvenile hormones could be useful for the control of aphids. 350 ppm doses (LD50 for aphid nymphs) of ZR-512, -515 and -777 applied 1 µl/egg, inhibit the egg hatching at 54.67% and 35.48% respectively on aphid predator ***Coccinella septempunctata*** L. (Coleoptera: Coccinellidae) (0-24 hr old eggs); ZR-777 did not effect (Kısmalı & Erkin, 1984). These researches also indicated the preference of the application of ZR-777 is more important for aphid control and its predatory; suggesting the choice of hormones in commitment of the pest control should carefully be considered for the possible impact of the useful insects.

The egg hatching percentages of 0.2, 1 and 5 µg/adult doses of ZR-777 on ***Tribolium castaneum*** Herbst (Coleoptera: Tenebrionidae) were found as 17.9, 1.3 and 0.0 respectively (Sing, 1994). It was reported that ZR-777 has a great potential for the control of this insect being a highly effective fertility curtailing agent.

In this study, egg hatching percentage is 100% in control groups. Embryonic death and egg hatching are related with applied concentrations at close range. At low concentrations (1-2 ppm) egg hatching rate is high, but it decreases at higher concentrations and gradually reaches to zero (Table 1, Figure 2). A correlation between the time period of egg hatching and treated ZR-512 concentrations was not found.

Morphological abnormalities on development

The morphological effects of the applied ZR-512 caused a number of abnormalities on embryonic development of ***M. desertus***. Different parts of the embryo such as unused quantity of vitellus, changes in development of mount parts, antennae, legs, cerci, body pigmentation and hair conditions. Seven days old ***M. desertus*** embryo selected for experiments is seen on Figure 3. Development of some body parts of an embryo in control group, near to hatch can be seen in hand drawn Figure 4, from ventral and dorsal views. The embryos completing development properly have achieved to hatch.

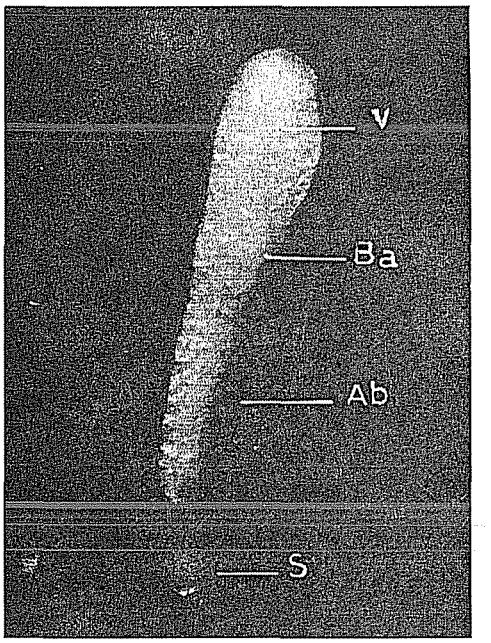

Figure 3. Seven days old ***Melanogryllus desertus*** embryo selected for ZR-512 application. V, vitellus; Ba, head; Ab, abdomen; S, cercus.

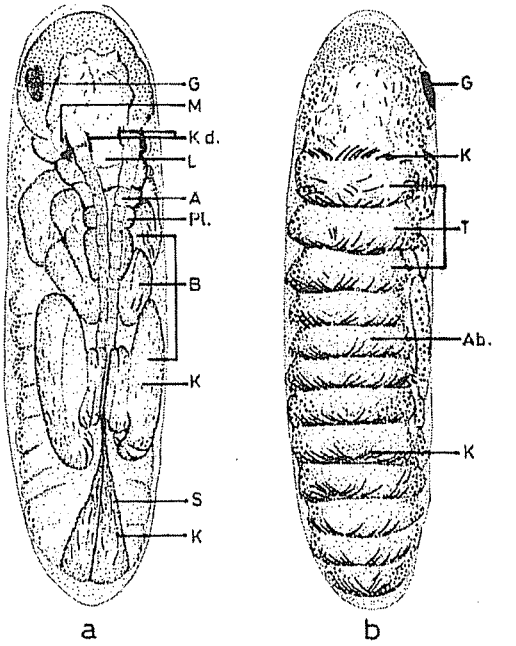

Figure 4. Ventral (a) and dorsal (b) appearances of a control embryo completed its development and achieved to hatch. G, compound eye; Kd, chorion hatcher teeth; M, mandibule; L, labrum; Pl, palpus labialis; A, antennae; K, hair; S, cercus; T, thorax; Ab, abdomen; B, legs.

The eggs after 7 days of the applications of concentrations sequentially 100 ppm, 1000 ppm and 10 000 ppm together with the normal and acetone controls are seen in Figures 5 a, b, c, d, e. In various concentrations of JHA that stops the egg hatching in different rates, the most evident and common abnormality is amount of unused quantity of vitellus (Figure 5 d, e). Hand drawn examples reflecting this abnormality are seen in Figure 6 from ventral and dorsal sides.

In general unhatched embryos are very small in size. Unused vitellus was remained on the top of the heads of embryos in different amounts (Figure 5, 6).

The embryos resembling to controls according to used amounts of vitellus (Figure 7) were also died because of they were unable to emerge from chorion or serosa (Figure 8). Large mandibules were found in side parts of the head. The special teeth (Figure 4, Kd) that are used for emerging from chorion were not seen. Antennae were changed in length. Some of their segments were distended, dark pigmented and not contained hair. Legs of embryos were smaller than those found in control groups. Short leg parts were swollen and were similar to buds. In some embryos, body pigmentation was observed as extremely developed manner, but in others not. Body hairs were also not seen. Many deformations as being smaller and leaf-like in shape were found in cerci (Figure 5, 6, 7).

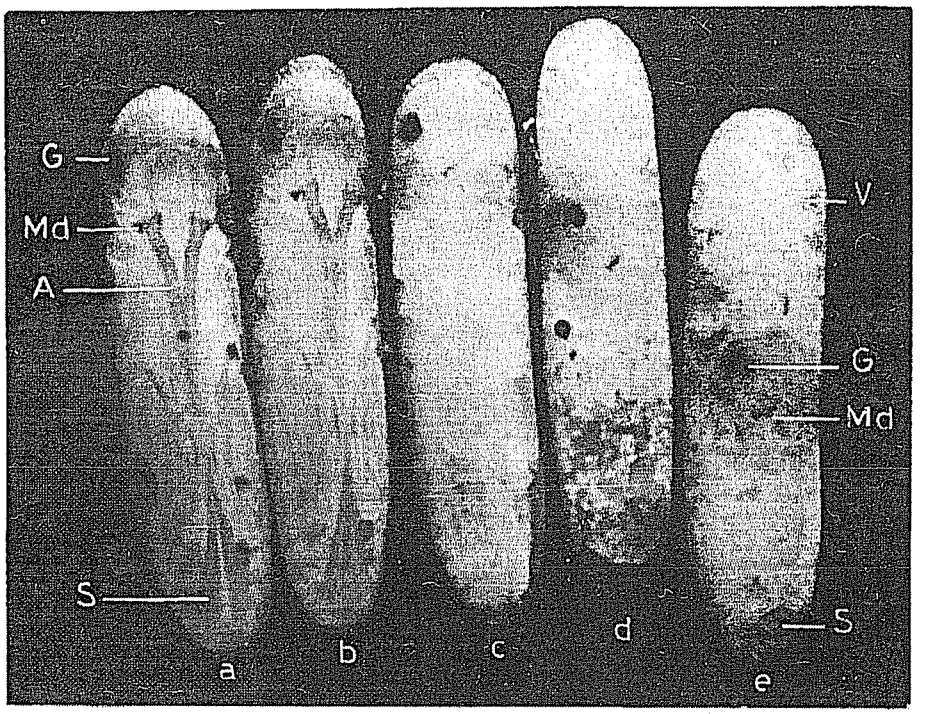

Figure 5. Abnormalities in ZR-512 treated embryos together with control groups. X 24.6. a) Normal control b) Acetone control c) 100 ppm ZR-512 d) 1000 ppm ZR-512 e) 10 000 ppm ZR-512 G, compound eye; Md, mandubule; A, antennae; S, cercus; V, vitellus.

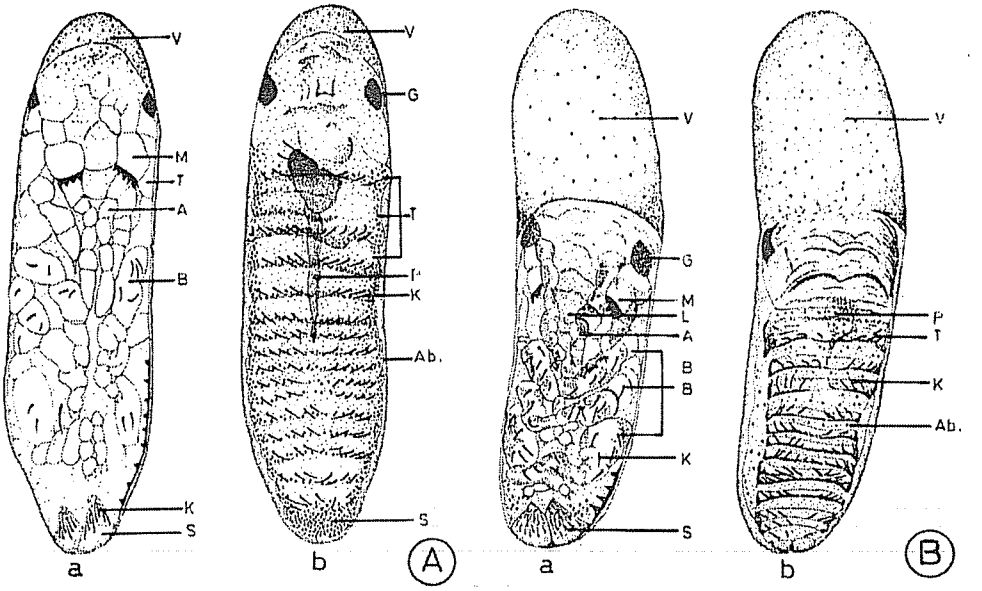

Figure 6. Hand drawn appearance of abnormal developed embryos in the hormone applied eggs, from ventral (a) and dorsal (b) views (A). Egg which has less, unused vitellus (B). Eggs whose vitellus has been used very little. G, compound eye; M, mandibule; L, labrum; A, antennae; K, hair; S, cercus; T, thorax; Ab, abdomen; B, legs; P, pigment; V, vitellus.

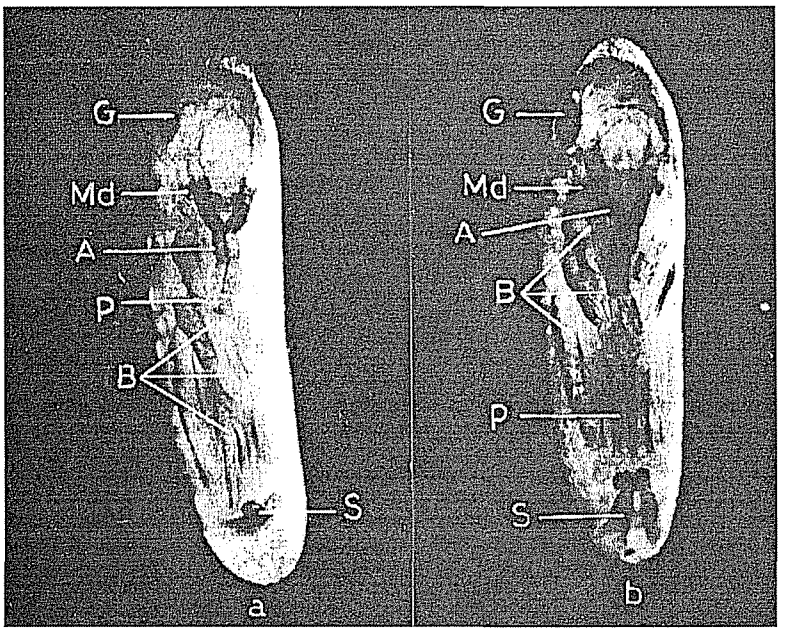

Figure 7. 2 ppm (a) and 4 ppm (b) ZR-512 applied eggs used all of their vitellus.
G, compound eye; Md, mandibule; A antennae; P, pigment; B, legs; S, cercus.

Abnormalities and spoils were demonstrated in embryonic development after several hormone applications (Ellis et al., 1970; Staal,1972, 1974). ZR-515 treatment on the eggs of ***M. desertus*** have also the similar effects (Geldiay et al., 1981). According to vitellus usage, body pigmentation and size of body and the legs of unhatched embryos, the results in ***M. desertus*** are similar to the abnormalities obtained in ***Schistoserca gregaria*** Forsk. (Orthoptera: Acrididae) (Novak, 1969) and ***Thermobia domestica*** (Rohdendorf & Sehnal, 1973). It is stated that after a short period of application on embryos of ***Thermobia domestica***, the mandibular teeth and differentiated eyes of embryos embeded in vitellus were appeared. But, these were not distinguished in the embryos of ***M. desertus***.

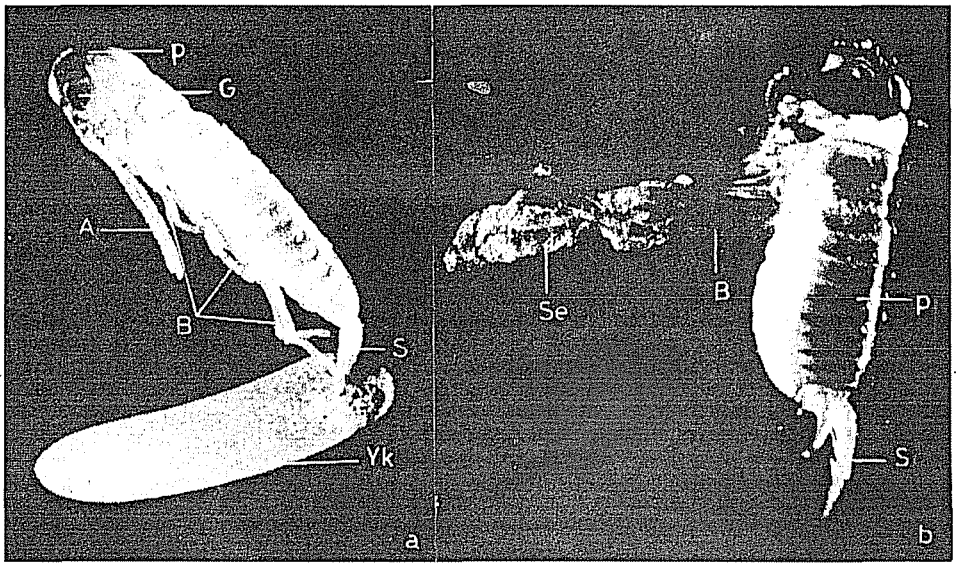

Figure 8. 4 ppm ZR-512 treated and died abnormal embryos. a) Died after emerging from chorion, b) Died because of failing to emerge from serosa. P, pigment; G, compound eye; A, antennae; B, legs; S, cercus; Yk, chorion; Se, serosa.

However, morphological abnormalities observed in antennae, cerci and legs of ***M. desertus*** were not found in the above mentioned insects. Kısmalı & Erkin (1984) indicated that ZR-512 applied some ***Coccinella septempunctata*** eggs, the embryos died, in the others, although some darkskinned larvae had developed, they were not able to hatch. It was informed by Hicks & Gordon (1992) that, fenoxicarb applied ***Choristoneura fumiferana*** adults laid their eggs, but they were completed their embryonic development abnormally, and not hatched.

As a result, ZR-512 is an effective hormone analogue on egg hatching and embryonic death for ***M. desertus***. When compared to the effects of ZR-515 on the same insect (Geldiay et al., 1981), inhibition concentration for 100% egg hatching was found as 30.83 ppm for ZR-512 and 45.65 ppm for ZR-515, while the concentration that has a 50% inhibitory effect is 5.957 ppm for ZR-512 and

4.225 ppm for ZR-515. The dose of ID50 ovicidal seems slightly less than those found for ZR-515, but still ZR-512 is an active compound having a potential to use for insect control.

Özet

Melanogryllus desertus **Pall. (Orthoptera: Gryllidae)'un yumurta açılması ve embriyonik gelişimi üzerine Juvenil Hormon Analoğu ZR-512' nin etkileri**

Melanogryllus desertus'un yumurta açılması ve embriyonik gelişimi üzerine Juvenil Hormon Analoğu ZR-512'nin etkileri araştırılmıştır. 1 µl aseton içinde ZR-512'nin farklı dozları yedi günlük erken blastokinesis evresindeki yumurtalara yüzeysel olarak uygulanmıştır.

Yumurta açılmasını yüzde yüz engelleyen etkili doz 30.83 ppm, % 50 engelleyici etkiye sahip konsantrasyon 5.957 ppm bulunmuştur. Yumurta açılmasında etkisiz konsantrasyon 1.384 ppm'dir.

Farklı ZR-512 konsantrasyonları embriyonik yapılarda örneğin; ağız parçaları, antenler, serkus ve bacaklar, kıllar ve vücut pigmentinin gelişiminde bazı bozukluklara neden olmuştur. Kullanılan vitellüs miktarında bazı farklar vardır. Sonuçlar ZR-512'nin ***M. desertus***'un yumurta açılması ve embriyonik gelişiminde etkili bir hormon analoğu olduğunu göstermiştir.

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