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THE RELATIONSHIP BETWEEN ANTIJUVENILE HORMONE (PRECOCENES) AND ENTOMOPATHOGENIC, *STEINERNEMA CARPOCAPSAE* TO NEMATODES INFECTIVITY AND ENERGY METABOLISM SUPPLY OF *SPODOPTERA LITTORALIS* (BOISD.).

ABD EL-KERIM IBRAHIM A. AND AHMED M. AZAZY

Plant protection Research Institute, Agricultural Research Centre, Dokki, Giza.

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Abstract

Laboratory investigation was carried out on 6th instar larvae of *Spodoptera littoralis* tissues which treated with precocenes, insect pathogenic nematode or precocenes-nematodes complex in order to clarify the infectivity of nematodes, mortality of *S. littoralis* larvae, the survival rate of nematodes infective juveniles (IJ) and energy consumption of the metabolic pathways, established in reserve materials of *S. littoralis* larvae. The obtained results of nematode-precocene combinations exhibited that larvae treated with 40 µg and 25 µg (PI+N) suffered mortality, reaching to 92% and 157.14%, respectively, compared to precocene treatment alone. This may due to enhanced infection by the nematode. This lethal effect was most probably caused by stressing metabolites, then blocking the process of their role in the self-defense, through metabolites targeting. A protein depression of about 81.57% was found in the larvae treated with 40 µg (PI+N) and of about 74.31% in those treated with with 25 µg (PI+N) compared to control treatment.

INTRODUCTION

It is known that antihormones from plants can compete with the most effective toxicants, of these, Precocenes, the natural products extracted from *Ageratum* spp., which have been shown to be cytotoxic to the corpora allata and preventing the production of juvenile hormone. The purpose of this study was to know parasitic nematodes compatibility with antijuvenile hormone (Precocenes) for (i) determining the key energy reserve compounds of IJ, (ii) determining the relationship between the consumption of energy reserve compounds and survival and infectivity of IJ via antijuvenile hormone.

MATERIAL AND METHODS

Insect colony: The larvae of the Egyptian cotton leaf worm, S. littoralis strain used in the present series of experiments were reared as a method adopted by Hegazi *et al.* (1977).

Nematodes culture: The entomopathogenic nematode, *Steinernema carpocapsae* was maintained and cultured in vivo as described by Dutky *et al.* (1964). On the last instar of the wax moth *Galleria mellonella*, nematode infective juveniles (IJs) were stored at 4-8°C und used within the first week of harvest.

Chemicals used: PI (7-methoxy-2,2-dimethyl-3-chromene) and PII (6,7-dimethoxy-2,2- dimethyl-3-chromene).

Applications of Nematodes and Precocenes: Precocenes dissolved in acetone were applied topically and individually at concentrations of 25 and 40 µg per 5 µl on the thoracic dorsum of test larvae, by means of a micro-applicator. Two groups (100 larvae / group) of 2-days old 6^{th} larval instar were used for each concentration of the tested compound. One group was used for treatment only with precocenes, while the other was treated with nematode infective juveniles (Ijs) of *S.carpocapsae* after its previously treated with precocenes. A third group of larvae was treated with nematode suspended in distilled water alone.

Each experiment was replicated 3-4 times (100 larvae each). Control experiments were run using larvae treated with acetone in case of precocenes or distilled water in case of nematode treatment alone and with combination of acetone and distilled water was successively in the case of preconcenes and nematode treatments.

Infectivity testing: The infectivities of the IJ were assayed by using the following two protocols, described by Bedding and Molyneux (1982) and Koppenhofer *et al.* (1998):

I- Exposure of 100 *S. littoralis* larvae to 5000 IJ on moist filter paper in petri dish , at 25°C for 4 days followed by assessing mortality. The dead larvae were dissected at the end of the 5th day after infection and number of nematodes was recorded.

II- Measurement of survival: Dead larvae were picked-up for nematode extraction. Recovery of nematode IJs in tissues was carried out by the baiting method using *G. mellonella* larvae. Survival of nematode IJs recovered was inspected by observing their

mortality in the water suspension and response to probing under a dissection microscope.

Biochemical analysis of insects: The larvae were homogenized in distilled water (4ml) and centrifuged at 4°C and 8500 rpm to remove particulate material. Main metabolites were quantitatively estimated as a total protein, carbohydrates and lipids. The metabolites were colourimetrically determined pursuing the techniques outlined by Dubois et al. (1955) for carbohydrates determination, Bradford (1976) for protein estimation and Knight *et al.* (1972) for lipid estimation.

Statistical analysis : carried out using a computer software package " Costat ", a product of cohort software Inc., Berkeley., California , U.S.A. Duncan's multiple range test was used to differentiate between means.

RESULTS AND DISCUSSION

The dynamics of reactions of *S. littoralis* 6^{th} larval instar toward *S. carpocapsae* and precocenes, revealed highly prominent changes. A drastic increase in the number of larval of *S. littoralis* mortality was found after precocene and nematodal treatment successively (92% relative increase in the 40µg PI treatment and 157.14% in the 25µg PI treatment). The Infective juveniles which stressed by precocenes reaveled a noticeable increase in survival rate of IJ treated with PI compared to those treated with PII, these effects were dose-dependent, Table 1. As shown in Table 2, larvae treated with precocene alone or precocene-nematode combinations exhibited decrease in total lipid, carbohydrate and mostly achieved with protein, reaching to (30.85, 82.47,81.57%), respectively with 40 µg (PI+N) and 40.02,62.81,74.31 %, respectively. In case of treatments with 25µg (PI+N) compared with control, the values were 14.77,60.83,60.25%, respectively with 40µg (PI+N) and 36.62,2.03,51.62 %, respectively with 25µg (PI+N), compared to PI. The latter application of PI+N indicated significant decrease in the three tested metabolites. In view of the above findings, it seems that the effectiveness of precocene/nematode related to the levels of energy reserve materials, (lipid, carbohydrates and protein) in the host (*S. littoralis* larvae), that supply energy consumption for *S. carpocapsae*. This may cause the inhibitory effect on haemopoiesis and consequently on the haemopoietic organs to produce the prohaemocytes. In the same time delaying or regress proteins could lower the number of cells available for two major immune reactions, encapsulation and phagoctosis, which made self-defence weakly and increased the efficacy of infective juveniles (IJ) of *S. carpocapsae*. In addition, it made the reserve materials more available for energy consumption of *S.*

carpocapsae. Yooko *et al.* (1992) found that *S. carpocapsae* nematode and /or its symbiotic bacterium, *Xenorhabdus nematophilus* interfere with the role of haemocytes in self- defence of *Agrotis segetum* larvae, suggesting that the supperession of the activation of prophenoloxidase may be due to making of the recognition protein, or by blocking of the process of prophenoloxidase activation (*lipopoly sacccharide*) and cause a suppression of their role in self defence. The presented findings agree with those obtained by Annadurai and Rembold (1993) showing mainly disappearance of a poly peptide profiles of adult female *Schistocerca gregaria* as a relative role in immunorecognition system and Tahany *et al.* (2001) who observed that the main phagocytic haemocytes (plasmatocytes-and granulocytes) quickly lose their ability to produce pseudopodia and can not spread. They suggested that the metabolites are targeting the haemocyte cell membrane or may be governed by hormonal secretion as mentioned by Shebl, (1979), who noted that precocenes acted indirectly on ecdysteroids that seems to be under stressing juvenile hormone secretion or corpora allata failure. Furthermore, the prothoracic glands (ecdysteroids secreation) can stimulate production of haemocytes in locust migratoria last nymphal instar (Hoffmann, 1970). Similarly, Rao *et al.* (1984) in *S. littura*. The obtained information provides a basic for increasing infectivity of infective juveniles (IJ) by antijuvenile hormone. But, insecticide might have served as stressors to suppress the infection by the nematode. However, not all pesticides were compatible with nematodes and each should be examined for compatibility, especially hormonal action as juvenoids (IGRs).

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Table 1. Mortality of *S. littoralis* 2 days old 6th instar larvae and survival rate of IJs of *S. carpocapsae* after treatment with precocenes alone and in combinations with nematodes.

Mortality of *S. littoralis* (%)				Survival rate % of Ijs/larva			
25 µg/insect		40 µg/insect		PII		PI	
PI+N	PI	PI+N	PI	40 µg	25µg	40 µg	25µg
90.00	35.00	96.00	50.00	89.32	90.61	91.57	93.33

Table 2. Effect of entomopathogenic nematodes (EPN), *S. carpocapsae* and precocenes treatment on main metabolites of S. *littoralis*, 6th instar larvae.

Status	Control	40 µg/insect		25 µg/insect	
		PI	PI+N	PI	PI+N
Lipid (g/l)	16.63 a	10.02 b	11.50 b	14.69 a	9.31 b
Carbohydrate (mg/dl)	15.57 a	6.07 b	2.73 c	5.91 b	5.79 b
Protein(g/dl)	36.4 a	16.88 b	6.71 d	28.14 b	9.35 d

F value for lipids = 35.061; for carbohydrates = 85.02; for protein = 348.06.

العلاقة بين مضاد هرمون الحداثة (بريكوسين) ونيماتودا التطفل (استشرنيما كاربوكابسا) على الغزو النيماتودى وعمليات التمثيل الغذائى لإطلاق الطاقة فى دودة ورق القطن (اسبودوبتيرا ليتورالس بوزيد)

ابراهيم على عبد الكريم - أحمد محمد عزازى

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقى - جيزة

لدراسة تأثير الإفراز الهرمونى بداخل جسم حشرة دودة ورق القطن على حيوية وفاعلية النيمانودا تم إستخدام مانعات هرمون الحداثة (البريكوسين) كأداة لتدمير هرمون الشباب فى يرقات العمر السادس لأنها أكثر حساسية للنيماتودا . حيث تم تطبيق مانعات هرمون الشباب بجرعات ٢٥ و ٤٠ ميكروجرام لكل حشرة ثم أعقبها المعاملة بالنيماتودا بتركيز ٥٠٠٠ فرد من النيماتودا على بعض الأفراد التي سبق معاملتها سطحياً بالبريكوسين . تم تسجيل أثر ذلك على حيوية النيماتودا وكفائتها وقدأدت المعاملة الى ذيادة نسبة الابادة للبرقات المعاملة بالبريكوسين ثم النيماتودا الى ٩٢٪ مع التركيز المرتفع و ١٥٧,١٤٪ مع التركيز المنخفض بالمقارنة بالمعامل بالبريكوسين فقط . كذلك تم دراسة أثر تعاقب المعاملتين على التمثيل الغذائى بداخل جسم الحشرة (الكربوهيدرات ، اللبيدات والبروتين) وقد أظهرت النتائج إنخفاض فى تركيز تلك المواد التى تستخدم لأنتاج الطاقة بالنيماتودا وعمل جهاز المناعة باليرقات خاصة البروتينات حيث وصل الأنخفاض الى ٨١,٥٧٪ مع التركيز المرتفع و ٧٤,٣١٪ مع التركيز المنخفض بالمقارنة مع اليرقات غير المعاملة .

معنى ذلك أن عمليات التثيل الغذائى وجهت لحصول النيماتودا على الطاقة على حساب عمل الجهاز المناعى بداخل جسم الحشرة مما يستوجب معه اجراء التجارب المعملية قبل إستعمال منظمات النمو الحشرية من مشابهات هرمون الحداثة الى جانب النيماتودا وذلك ضمن المكافحة المتكاملة .