



A Study on the Effect of Some Bacterial Isolates on the Land Snail *Monacha Obstructa*, (Mollusca, Gastropoda) As a Biological Control

دراسة عن تأثير بعض عزلات من البكتريا علي القوقع الأرضي هوناكا أوبستركتا (رخويات : بطن قدهيات) كهمقاومة بيولوجية.

Gehad Mohamed Nael Ahmed El-Said Mousa Aboul-Nasr

جهد محمد نائل أحمد السيد موسى أبوالنصر

Nasser Abd El-Latif El-Shimy, Torkia Abul-Magd Mohamed, Safaa Megally Ali

ناصر عبداللطيف الشيمي، تركية أبوالمجد محمد، صفاء مجلى على

Abstract:

The present work aimed to study the molluscicidal effect of three bacterial isolates of *Bacillus thuringiensis*, (Bt-122, Bt-123 and Bt-NCCB) which were introduced via soil and food against the land snail *Monacha obstructa* (The clover glassr snail). This snail is considered the most serious agricultural pest causing substantial damage to different crops at Assiut governorate. This work also aimed to study of the differences in haemolymph cellular (haemocytes) and histological changes in the digestive gland of the land snail *M. obstructa* before and after treatment with the bacterial isolates. The only effective concentration was 1×10^{20} cfu/ml in all bacterial isolates. The mortality percentage greatly increased after four weeks of exposure to isolate Bt-122 1×10^{20} cfu/ml followed by isolate Bt-ncb 1×10^{20} cfu/ml and Bt-123 1×10^{20} cfu/ml which gave the same value. The isolate Bt-122 1×10^{20} cfu/ml gave the highest mortality rate and higher applied concentration of it (1×10^{25} cfu/ml) gave the highest percentage of mortality. Light microscopic investigations were carried out to study the differences in the haemolymph (haemocytes) of the land snail *M. obstructa* before and after treatment with the three bacterial isolates. Three main haemocyte categories could be distinguished namely: hyalinocytes, agranulocytes and granulocytes according to the presence or absence of granules, the ability of the cytoplasm and granules (if present) to stain as well as the nucleic/cytoplasmic ratio. Several differences were observed in the morphology, differential count and nucleic/cytoplasmic ratio (N/C ratio) of the haemocytes in untreated and treated snails. Hyalinocytes have the highest N/C ratios in untreated and treated snails. The predominant type of haemocytes counted in untreated snails after one and four weeks, treated snails with Bt-122 1×10^{25} cfu/ml after one week, treated snails with Bt-123 1×10^{20} cfu/ml and Bt-NCCB 1×10^{20} cfu/ml after four weeks was agranulocytes with acidophilic cytoplasm (43.13, 54.4, 66.8, 51, 46.4% of the counted cells). While, agranulocytes with basophilic cytoplasm in treated snails with Bt-122 1×10^{20} cfu/ml, Bt-123 1×10^{20} cfu/ml and Bt-NCCB 1×10^{20} cfu/ml after one week and in treated snails with Bt-122 1×10^{20} cfu/ml and Bt-122 1×10^{25} cfu/ml after four weeks were the predominant haemocyte type (55.5, 86.2, 55.6, 51.6, 36.4% of the counted cells). The morphological features of the haemocytes types remarkably varied from being regular spherical, oval, polygonal and have few pseudopodia in untreated snails to be rod, spindle and irregular shaped cells in treated snails with the Bt isolates. In treated snails with Bt cells had many cell-fragments and undergo cell-division. Highly vacuolated cytoplasm, vacuolated cells, destructed cells, formation of numerous long filopodia and pseudopodia were detected. Cells phagositized bacterial cells and cells that had a transparent material and faintly stained cytoplasm were recognized. By comparing (N/C) of each cell type in untreated and treated snails basophilic haemocytes were significantly increased in treated snails with Bt-122 1×10^{20} cfu/ml and in treated snails with Bt-123 1×10^{20} cfu/ml after one week and in treated snails with Bt-122 1×10^{20} cfu/ml, in treated snails with Bt-123 1×10^{20} cfu/ml and in treated snails with Bt-NCCB 1×10^{20} cfu/ml after four weeks. However it significantly decreased in treated snails with Bt-122 1×10^{20} cfu/ml after one week. The digestive gland represents the main target organ for molluscicide impact. Thus, the present work studied the histological structure of the digestive glands in order to determine the changes that were occurred after exposure to different spore suspensions concentrations of the three used isolates of Bt. Four different types of cells were observed in the digestive gland of snails under study: digestive cells, calcium cells, excretory cells and thin cells. The present observation showed that the used Bt isolates affected their structure. The lumens of most digestive tubules were filled with



secretory materials. Complete degeneration of some digestive tubules cells was observed and the intertubular connective tissue among most tubules showed great destruction. There was great vacuolization of digestive tubule cells. All these changes in treated snails indicate that the use of Bt isolates affected the immune response mediator (hemocytes) and the key organ of metabolism (digestive gland) and thus, it provides an effective biological control agents against land snails.

المخلص:

يعتبر القوقع الأرضي مونكا اوبستركتا (قوقع البرسيم الزجاجي) من أخطر الآفات الزراعية لما يسببه من أضرار بالغة في العديد من المحاصيل في محافظة أسيوط ولذلك هدفت الدراسة الحالية لمقاومة هذا القوقع بيولوجيا باستخدام ثلاث عزلات بكتيرية باسيلوس ثرينجينسيس باستخدام طريقة غمر أوراق الخس و خلط التربة بالعزلة البكتيرية المختبرة في نفس الوقت. كما هدفت أيضا هذه الدراسة إلي تحديد الفروق بين تركيب الهيموليمف (خلايا الهيموليمف) وكذلك تحديد التغيرات الهستولوجية في الغدة الهضمية قبل و بعد التأثير بالعزلات البكتيرية. و قد وجد أن التركيز الفعال فقط هو 1×10^{20} ml/cfu* في جميع العزلات البكتيرية. وقد زادت نسبة الموت زيادة كبيرة بعد أربعة أسابيع في القواقع المعاملة بالعزلة Bt-122 العزلة أن وجد وقد. القيمة نفس أعطنا اللتان Bt-123 1×10^{20} cfu/ml و Bt-NCCB 1×10^{20} cfu/ml العزلة تليها 122×10^{20} cfu/ml أجريت وقد. الموت من نسبة الأعلى هو كان (1×10^{25} cfu/ml) ذلك من الأعلى التركيز تطبيق وعند موت نسبة أعلى أعطت 1×10^{20} cfu/ml دراسة باستخدام المجهر الضوئي لدراسة الاختلافات في خلايا الهيموليمف. فوجدت ثلاثة أنواع رئيسية من خلايا الهيموليمف وهي كالاتي خلايا زجاجية. خلايا غير محببة و خلايا محببة و ذلك طبقا لوجود أو عدم وجود الحبيبات و قابلية الحبيبات للاصطباغ (ان وجدت حبيبات) و نسبة النواة للسيتوبلازم. وقد لوحظت العديد من الاختلافات في الشكل الخارجى. العدد النوعى و نسبة النواة للسيتوبلازم في كل من القواقع غير المعاملة أو المعاملة. وقد كانت نسبة النواة للسيتوبلازم أعلى ما يمكن في الخلايا الزجاجية في القواقع غير المعاملة أو المعاملة. كما كانت الخلايا عديمة التحبب الحامضية هي النوع السائد في القواقع غير المعاملة قبل و بعد أربعة أسابيع و القواقع المعاملة بالعزلة Bt-122 1×10^{25} cfu/ml بعد أسبوع واحد ، والقواقع المعاملة بالعزلة Bt-123 1×10^{20} cfu/ml و Bt-NCCB 1×10^{20} cfu/ml بعد أربعة أسابيع و كانت النسب كالاتي:- 43% 54 43% 51 66% 8

على الترتيب