THE ROLE OF SECONDARY METABOLITES PRODUCTS, IN THE SUSCEPTIBILITY OF POTATO PLANTS TO TUBER MOTH

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Abstract

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) is considered the most serious potato insect pest. Varied categories of infestation were observed on different potato cultivars. Potato varieties resistant to insect pests are considered great source of potentiality. Nicola potato cultivar, however was reported as the least performance variety to PTM infestation. Sponta cultivar demonstrates with moderate infestation levels and Arinda cultivar declares the highest level of PTM infestations. The potato plants produced certain metabolic substances that had its role in impediment or made the plants not favorable to the PTM larvae. Phenolic compounds and glutamic acid of the secondary plant metabolites were determined and approved to have certain action on the PTM infestation. The chemical analysis revealed that potato cultivar that demonstrates high content sap of total phenol, high content of conjugated phenol, lowest content of free phenol and lowest content of glutamic acid harbored the least level of PTM infestations. That will give us ideal explanation why Nicola cultivar is considered as the least susceptible in PTM infestations. These circumstances were accomplished with it, and vice versa i.e. Arinda cultivar demonstrates the highest level of PTM infestations. The idea behind this research is to promote biotechnological approaches that can selectively increase the amounts of defense compounds in crop plants, thereby reducing the need for costly and potentially toxic pesticides. Other approach is to promote for importing and/or planting the potato cultivars that perform with good indication of having adequate amounts of these metabolic substances just to minimize the chemical insecticides as far as possible.

INTRODUCTION

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera, Gelechiidae) is considered the most serious potato insect pest. Potato losses due to PTM can be devastating as without control measures tuber infestation can reach 100% (Raman *et al.*, 1994). Plants produce a great variety of organic compounds that are not directly involved in primary metabolic processes of growth and development. Many classes of secondary plant substances demonstrate toxic or antimetabolic effect on target insects. A common feature of these compounds is having a chronic rather than an acute toxicity on insects and their effects are less dramatic than synthetic insecticides. Natural products appear to function primarily in defense against predators and pathogens. According to Schoonhoven, 1982, Rosenthal and Berenbaum 1991, Sharma and Norris, 1991 and Estruch *et al.* 1997, most natural products can be classified into three major groups: terpenoid, alkaloid, and phenolic compounds. Alkaloids are synthesized principally from amino acids. These nitrogen-containing compounds protect plants from a variety of herbivorous pests. Various alkaloids are toxic to insects or function as feeding deterrents. The steroid alkaloid α-solanine, a cholinesterase inhibitor found in potato tuber, is the trace toxic constituent. Tannins, lignans, flavonoids, and some simple phenolic compounds serve as defenses against herbivores. Biotechnological approaches can selectively increase the amounts of defense compounds in crop plants, thereby reducing the need for costly and potentially toxic pesticides.

Phenolic compounds are a large, heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom. The antioxidative properties of phenolic acids play an important role in the antioxidative defense mechanisms of biological systems. Potato tubers contain a large number of phenolic compounds. Some of them appear in free and some in bound form. The largest portion of phenolic compounds is found in potato in the skin and the periderm layer next to it. There is about ten times as much phenolic compounds in the peel as in the flesh of the potato tubers (Malmberg and Theander, 1984, Strube *et al.* 1993 and Macheix and Fleuriert 1998).

Glutamic acid is one of chemicals isolated from potato plants, was reported to be an oviposition localizer. Asparagine and glutamine are present in approximately equal amounts, and together constitute about one-half of the total amino acids (LeTourneau, 1956, Meisner *et al.*, 1974 and Valencia, 1985).

MATERIALS AND METHODS

1- Potato cultivars Three potato cultivars were examined for the PTM infestation in stores at the International Potato Center (CIP), at Kafr El-Zayat, Gharbia Governorate during June, July and August of season 2003. Three commercial potato cultivars: Nicola, Spunta and Arinda, were chosen to determine the percentage of tuber infestations, the phenolic compounds and the glutamic acids contents in each of them. At a nawala store, 200 kg of each potato cultivar were inspected carefully and stored in heaps after covering with rice straw for tree months. A sample of 100 tubers replicated three times was selected randomly for inspection three times at 30 day intervals. Infestation and gallery numbers for each cultivar were recorded.

Other samples of potato tuber for each cultivar were sent to the Plant Pathology Research Institute laboratory to determine the following:

1- Phenolic compounds Phenolic compounds (free, conjugated and total phenols) for each cultivar were determined using colorimetric method as described by Snell and Snell (1953). Five gram of fresh sample were cut into small portions and immediately stored in 25ml of 95% ethanol in dark bottles, then kept under dark conditions at room temperature. Ethanolic extractions were subjected to air current at room temperature till dryness, and then they were quantitatively transferred into 10 ml of 10% isopropanol and stored in vials at 1°C.

1-1 Determination of free phenols Free phenols were determined by adding 1ml of the extracted sample to 1ml distilled water, 1ml Folin-Denis reagent and 3 ml $Na_2 CO_3$ 20% (w/v) in a sterilized test tube. The mixture was completed to 10ml using distilled water, and the color density was detected spectrophto-metrically at 520 nm. The free phenols were determined as mg catechol/ gm dry weight from the standard curve of catechol.

1-2 Determination of the total phenols One ml of the extracted sample was treated using 0.25 ml HCl and boiled in a water bath for 10 minutes then cooled. The mixture was completed to 10 ml using distilled water and the color density was detected at 520 nm.

1-3 Determination of conjugated phenols Conjugated phenols were determined by subtracting the amount of free phenols from total phenols.

2- Determination of glutamic acids For the separation of glutamic acids, one thin layer chromatography was used for this determination by Hptic apparatus. Samples of each potato cultivar were used at 0.05 ml and spotted on chromatograms. Reference of the amino acid was prepared and spotted on the same chromatograms containing the unknown samples (extracts). The used solvent composed of n-butanol: glacial acetic acid: water (4:1:2"v"v"v) according to (Block, 1958). Chromatograms were run twice in the same solvent, each for 24 hours (Ambe and Toppel, 1961). The spots were made visible by means of 0.2% ninhydrin in acetone (Smith, 1958). Color development was achieved by heating the dried chromatograms in an electric oven at 70°C for 15 minutes. Identification of glutamic acid on the developed chromatograms was carried out to their Rf value, color reaction and Rf value of the known amino acid. The quantitative determination was achieved automatically, by Hptic depending on the color density that was measured at 570 nm.

The difference between different cultivars was determined using Duncan Multiple Range Test (DMRT) at 0.05% level. Function correlation between tuber infestations and Phenol and Glutamic acid were determined by Minitab Program.

RESULTS AND DISCUSSION

1- Potato cultivars and PTM infestations: The degree of susceptibility to PTM infestation of the three tested potato cultivars was shown in Table 1 and Figure 1, expressed as percentages while the intensity of PTM infestation represented as gallery numbers in August, 2003. When the analysis of variance worked out it yielded significant differences between the tuber infestations patterns (P-value = 0.000), as well as between the gallery numbers (P-value = 0.004) for the different potato cultivars. Nicola cultivar recorded minimum tuber infestation (24.66), while Spunta cultivar recorded infestation of (32.66) and Arinda harbor the highest tuber infestation (42.66) at the 3rd inspection. Also, these cultivars recorded alternatively gallery numbers of 45.66, 61.33 and 89. There was a positive correlation between the tuber infestation and the gallery numbers (r. = 0.101).

2- The secondary metabolic products: The careful examination of the data in Table 2 and graphically in Fig. 2 revealed that different components of the intended materials associated with potato cultivars that believed to have relation with the mechanism of performing varied degrees of resistance to the PTM infestation. Also, Figure 2 showed the relation between PTM infestation and the secondary metabolic products of the different potato cultivars in August 2003.

Nicola cultivar, however, demonstrates the least figures (13.77 infested tubers) it got the highest total phenol (15.71 mg), highest conjugated phenol (13.94 mg), the least amount of free phenol (1.78 mg) and least amount of glutamic acid content (0.86 mg).

On the other hand where Arinda cultivar represented the highest tuber infestation (24.22 infested tubers), it was found that the total phenol was in minimum performance level (9.70 mg), the conjugated phenol also, recorded minimum amount of (6.10 mg), in the same time the free phenol recorded highest of (3.60 mg) and so the glutamic acid was in the highest performance recording (0.98 mg).

Table 1. Mean averages of infested tubers and gallery numbers on different potato cultivars in stores at the summer season of 2003.

Cultivar	1st Sorting		2nd Sorting		3rd Sorting	
	Tubers infest.	Gallery no.	Tubers infest.	Gallery no.	Tubers infest.	Gallery no.
Nicola	1.66	1.66	15	25.66	24.66	45.66
Spunta	2.66	3	23	33.33	32.66	61.33
Arinda	3.66	4	26.33	41	42.66	89

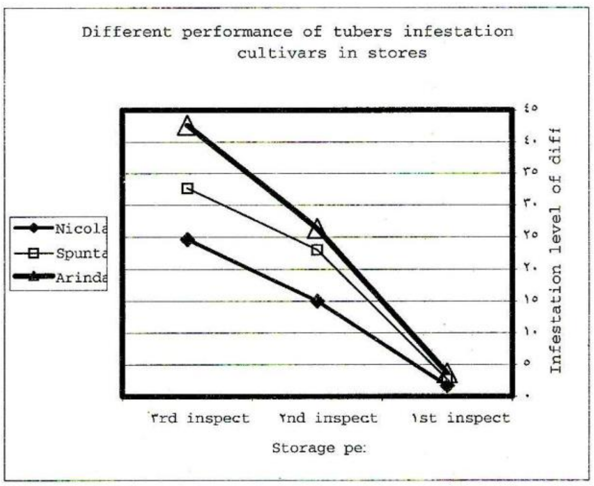

Figure 1. The PTM infestation during the three inspections for the different potato cultivars in August 2003.

Table 2. Determination of certain metabolic products (in mg) associated with the tested potato cultivars at the Plant Pathology Research Institute laboratory in August 2003.

Cultivars	Total phenol	Free phenol	Conjugated phenol	Glutamic acid
Nicola	15.72	1.78	13.94	0.86
Spunta	11.79	2.97	8.82	0.93
Arinda	9.70	3.60	6.10	0.98

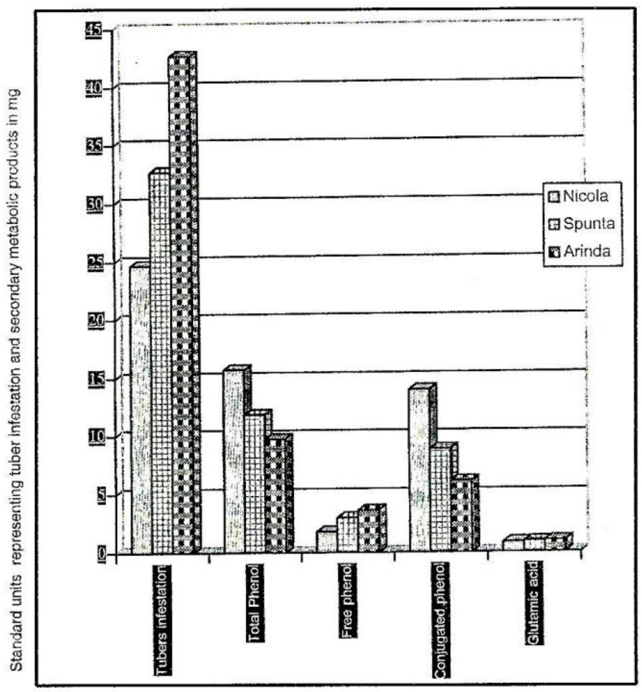

Figure 2. The relation between PTM infestation and the secondary metabolic products of the different potato cultivars in August 2003.

As Spunta cultivar ranks second when tuber infestation was considered (19.44). The total, free, conjugated phenol and glutamic acid amounts were in between the Nicola and Arinda cultivars.

The data related to glutamic acid showed that the increasing in corresponded with positive inner earring the tuber infestations and vice versa the minimum glutamic acid content the minimum tuber infestation was documented. The highest content of glutamic acid was found in Arinda followed by Sponta and Nicola cultivars.

In general, it could be concluded, however, those potato cultivars had the high concentration of the total phenolic comp ound and high concentration of the conjugated phenols demonstrates distinct susceptibility to PTM infestations. Also, potato cultivars that had the least concentration of these free phenols and least concentration of glutamic acid were not favorable to the PTM infestations. While potato cultivars with low contents of the total phenolic compound and low concentration of the conjugated phenols, high content of free phenol and high content of glutamic acid were favoring the PTM infestations.

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دور منتجات التمثيل الغذائى الثانوية لقابلية نبات البطاطس للإصابة بفراشة دودة درنات البطاطس

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تعتبر حشرة فراشة درنات البطاطس من أخطر الحشرات على محصـول البطـاطس وتسبب خسائر جسيمة للمحصول، ولقد شوهد تباين لدرجة وشدة الإصابة بالحشرة على أصناف البطاطس المختلفة. وأن ميزة مقاومة النبات للآفة تعتبر عامل هـام لتقليـل الإصـابة مقارنـة بالأصناف الحساسة للإصابة والتى تستدعى توافر وسيلة مكافحة أخـرى ذات تكلفـة أضـافية وكثيرا ما تكون ضارة بالبيئة فى حالة اللجوء للمكافحة الكيماوية.

ولقد لوحظ أن الصنف نيكولا قد سجل أقل إصابة بالآفة وصنف سبونتا فكان ذو إصابة متوسطة، أما الصنف أريندا فكان حساسا للإصابة بالآفة.

ووجد أن أصناف البطاطس تنتج مواد ثانوية خاصة نتيجة العمليات الحيوية المختلفـة بكميات متباينة تستخدم فى الدفاع ضد المتغذيات الحشرية المختلفة وتؤدى إلى تقليل أو الحد من الإصابة أو تجعل النبات غير مفضل لتغذية الآفة أكثر من نبات آخر ينتج كيماويات بدرجة أقل أو مختلفة، ومن هذه الكيماويات الثانوية تم تحديد المركبات الفينولية وحمض الجلوتاميك كمـواد محددة لتباين الإصابة بالفراشة.

أثبت التحاليل الكيماوية أن أصناف البطاطس التى تحتوى كمية عالية مـن الفينـولات الكلية والفينولات المرتبطة وفى نفس الوقت لها أقل محتوى من الفينولات الحرة وأقل محتـوى من حمض الجلوتاميك وجد أن هذه الأصناف كانت مسجلة أقل إصابة بالآفة وهذا يعطينا تفسير تميز الصنف نيكولا لأقل إصابة بالآفة حيث تتوافر به هذه الملابسات والعكس صحيح حيث وجد أن الصنف أريندا كان أكثر حساسية للإصابة حيث محتواه من المواد المحثة للإصابة كانت على النقيض مع الصنف نيكولا أما الصنف سبونتا فكان فى مرحلة وسطية.

وإن المغزى من هذا البحث هو الترويج لاتجاه التقنية الحيوية والتى قد تعمـل علـى زيادة كمية هذه المواد الدفاعية فى النبات وبالتالى تؤدى إلى تقليل الحاجة لاسـتعمال المبيـدات السامة والمكلفة فى نفس الوقت.

المغزى الآخر ممن هذا البحث هو الترويج لاستيراد وزراعة أصناف البطاطس والتى تحوى على كميات كافية من هذه المواد الثانوية أيضا بغرض تقليل الإصابة والاعتمـاد علـى المبيدات الكيماوية والتى تستخدم على محصول غذائى مثل البطـاطس عـلاوة علـى مشـاكل المبيدات المتعددة على البيئة .