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Macromolecular abnormalities in the adults of the potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) induced by larval feeding on irradiated potato tubers

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ABSTRACT

Potato tubers were irradiated with different doses of gamma irradiation (50,100 and 200Gy), and fed by the larvae of *Phthorimaea operculella* (Lepidoptera : Gelechiidae), to measure the effect of larval feeding on some biochemical components of the adult stage. The data indicated that the change in larval diet (tubers) due to irradiation led to some changes in such biochemical components of the adult. Irradiation increased phenolic content and oxidation capacity of potato tubers, while vitamin C was significantly reduced. Vitamin C content of the adult was not significantly changed at most doses. From the nutritional point of view, this means that the decrease in a nutrient in the diet not necessitate corresponding suppression in the feed insects. Acid and alkaline phosomonoesterases activities and trehalose content of the adult were significantly depressed. Such depression was related to the increased phenolic content of potato tubers after irradiation. This provides an evidence that irradiation of potato tubers could leads to products which might be toxic to insects and able to change in the diet (tubers) component that fed by the larvae. It is interesting to realize that ionizing irradiation inactivate pathogens or directly killed store insect stages, but also could control reinfestation of pest by producing changes in nutrients of potato tubers, and increasing toxic compounds, resulting in abnormalities which could extent to the adult stage and reduce its reproductive capacity.

Key words: *Phthorimaea operculella*, potato tubers, gamma radiation, macromolecular components.

INTRODUCTION

Larvae are actively feeding stage of holometabolic phytophagous insects; they are able to select the diet that will provide the best development. Nutritive value of food and the presence of secondary plant metabolites or allelochemicals in host plants determine performance of phytophagous larvae **(Mattson and scriber, 1987),** titer of detoxification enzymes **(Lindroth *et al.*, 1991 and Amin, 2007)**, and even reproduction of adults (**Fernandes-Da-Silva and Zucoloto, 1997).** The potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) is a major pest of potatoes worldwide **(Fenemor,e 1988).** Under storage, Potato tubers are the most vulnerable to infestation with such pest. During crop growth, the larvae mine into foliage while some may mine into stems causing entire terminals to die and resulting in significant yield losses. Conventional control methods are not very successful because the larvae pass a major portion of their life inside the tubers (**Haiba, 1990).** Ionizing radiation, e.g. gamma rays, X-rays or electrons, can be used to extend the life of certain fruits and vegetables by delaying ripening or killing

moulds, and for the control of insect infestation of grains and other stored products. It is also a safer and cheaper control method (**Elvin, *et al.* 1974; Henry, 1998 and Farkas, 2006).** It is interesting to know that feeding of potato tuber moth larvae on irradiated potato tubers, not only affect the developmental time and survival rate of the larvae, but also affect fecundity and fertility of the adult {**Rananavare *et al.*, (1991); Haiba, (1994 and 2000) and Saour *et al.*, (1999)}** Mechanism by which irradiated plants could induce such effects are not well established. However irradiation could change nutritional compounds such as sugar and vitamins of some plants **(Joshi *et al.*, 1990 and Fan *et al.*, 2003).** So that studies must be performed to explore the effects on extended adult insects**.** Feeding of tuber moth larvae on irradiated potato tubers could affect adults main metabolites **(Haiba and Abd EL-aziz, 2008).** From the nutritional point of view, are the changes in some nutritional compounds such as vitamin C in irradiated potato tubers, encountered by the same changes in adults fed as larvae on these plants? Is the reduction of reproductive capacity of adults could be due to the effects on vitamin C or phosphomonoestrases? Some authors {**Vanderzant and Richardson, (1963) and Moursy, (1995)}** reported the importance of L-ascorbic acid for reproduction in insects**.** Could irradiation increase toxic compounds on potato tubers such as phenolic content which may be implicated in the effect on adults of potato tuber moth? Trying to answer these questions, potato tubers were irradiated with different doses of gamma irradiation (50-200 Gy) and offered to larvae of *Ph. operculella* then some of biochemical components of the emerged adults were estimated.

MATERIAL AND METHODS

The potato tuber moths *Ph. operculella* (PTM) obtained from Nuclear Research Atomic Energy Authority, were reared in the laboratory of Entomology Department, Benha University, Faculty of Science. The PTM were reared on potato tubers (*Spunta variety*) after being cleaned from dust and parasites. Thereafter, the tubers were infested with PTM and kept in wire cages at room temperature. A thin layer of clean sand was furnished on bottom of cages for pupation **(El-Sinary, 1995).** Four groups of newly emerged adults (20 males and 20 females) of PTM were kept in four cages containing 500 gm of irradiated potato tubers (*Spunta variety*) with doses 0, 50, 100, and 200 Gy to lay their eggs on the tubers. Experimental analyses were carried out on the whole body content of unmated newly moths emerged from larvae fed on the non-irradiated and irradiated potato tubers.

Potato Irradiation was carried out through the model 3500 Gamma Cell Irradiation Unit (^{60}Co source) installed in the Middle Eastern Regional Radioisotope Center for the Arab Countries with a dose rate 1.3 rad/Sec.

Extraction and determination of total phenolics:

Ten grams of potato tuber were washed with distilled H_2o and placed in an oven to dry at 45C^o for 5 days. Then they were grounded in an electric grinder into fine powder. Extraction was performed as described by **Kähkönen *et al.*, (1999)** using 80% aqueous methanol. The amount of total phenolic in extracts was determined by Folin-Ciocalteu method as modified by **Singlton and Rossi (1965).** Phenolic content was expressed as mg gallic acid per gm dry weight of the original sample (mg GA\g . dw).

Preparation of tissues for analysis:

Adult moths or potato tubers were homogenized in distilled water (10 moths or 1 gm potato tuber/ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at

5 C^{o} in a refrigerated centrifuge. The deposits were discarded and supernatant was kept in a deep freezer at till use.

Analytical producers:-

Analysis of Ascorbic acid or vitamin C in adults of potato tuber moths or potato tubers was performed as recommended by **A. O. A. C. (1975)** method using 2,6- dichlorophenol-indophenol dye in the direct colorimetric determination. Trehalose was determined according to **Crompton and Brit, (1967)** using glucose as standard. Phosphomonoesterases (acid and alkaline) activities were measured as described by **Powell and Smith, (1954)** using disodium phenylphosphateas substrate. Phenoloxidase activity assayed according to a modification of **Ishaaya, (1971)** using catechol as the substrate. Absorbance was recorded after 1 min from the initiation of the reaction. Spectophotometer (Spectronic 1201, Milton Roy Co., USA) was adjusted at 405 nm against sample blank. For peroxidase assay, the method of **Vetter *et al.*, (1958)** was followed with some changes in the quantities of the reaction mixture. Hundred microliters of sample (moths or potato tubers homogenate) were added to 1 ml buffer (0.1 M phosphate-citrate, pH 6.5), and 100 µl of O-phenylenediamine (1%). The reaction was initiated by 100 µl of H_2O_2 and allowed to proceed for 5 min. at 30 C°. The absorbance was recorded as in case of phenoloxidase assay.

Statistical analysis:

All experiments were replicated 3-4 times (moths or potato tubers homogenates), and results of biochemical determinations were pooled from triplicate determination. To examine the effects of both adult sex and irradiation dose on the determined biochemical components, the two way ANOVA was used. Means and standard deviations were obtained using Costat statistical software. Means were tested for significance by the one way analysis of variance (ANOVA). When the ANOVA statistics were significant (P< 0.01), means were compared by the Duncan's multiple range test.

RESULTS

Table (1) shows the effect of larval feeding on irradiated potato tubers on *Ph. operculella* adults acid and alkaline phosphomonoesterases (Alkpase and Acpase). Alkaline phosphomonpesterase activity was decreased in both males and females as compared to control. Acid phosomonoesterase activity decreased significantly as compared to control at all irradiated doses. It is not affected in males except at 50 Gy irradiated dose where it was slightly decreased.

Table (1): Activities of acid and alkaline phosphomonoesterases enzymes (mean ±SD) of newly emerged females and males of *Ph. operculella* fed as larvae on irradiated potato tubers.

Dose in Gy	Alkaline phosphomonoesterases (*U x 10^3 / gm/**b.wt)		Acid phosphomonoesterases (U x 10^3 / gm/b.wt)	
	Female	Male	Female	Male
Control	503.3±10.4^{a}	448.3±11.2^{a}	53.2±1.26^{a}	59.7±1.5^{ab}
50	336.7±11.6^{c}	313.3±7.8^{b}	54.3±1.57^{b}	55.8±0.9^{c}
100	292±10.6^{d}	187.7±5.9^{c}	41.7±1.5^{c}	61.7±2.1^{a}
200	436±13.5^{b}	206.3±5.5^{c}	23.7±0.6^{d}	57.4±0.5^{b}

Vertically means bearing different letters are significantly different at P< 0.01.

*U= unit of enzyme activity which is the amount of enzyme hydrolyze 1µ mole of p/nitrophenyl / minute at 37C° and pH 9.8 or 4.8 for Alkpase and Acpase, respectively. ** b.wt = body weight

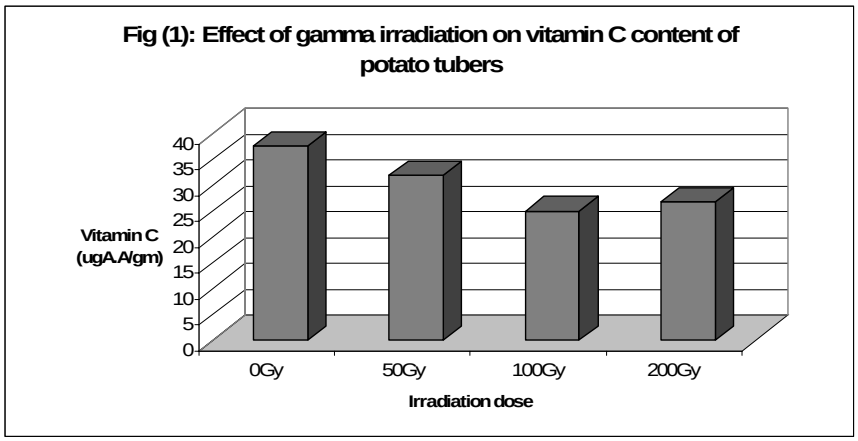
Another components which might be relates to the effect on reproduction of adults are trehalose and vitamin C. Table (2) indicated that although vitamin C in adults was not affected by potato tubers irradiation, gamma rays generally reduced moths trehalose content. Trehalose content equal to 14.65, 11.06, 9.78 and 9.51mg/g.b.wt for control females, and those fed as larvae on irradiated potato tubers 50,100 and 200 Gy, respectively.

Table (2): Trehalose and vitamin C (means ± SE) content of newly emerged females and males of *Ph. operculella* fed as larvae on irradiated potato tubers.

Dose in Gy	Trehalose (mg/g.b.wt)		Vitamin C(µg *A.A./ g.b.wt)	
	Female	Male	Female	Male
Control	14.65±0.45**a**	15.85±0.74^{a}	60.96±0.9^{a}	85.2±3.2^{a}
50	11.06±0.12^{b}	13.64±0.29^{b}	65.4±2.1^{a}	80.4±1.7^{b}
100	9.78±0.09^{b}	16±1.5^{a}	61.2±2^{a}	85.69±2.5^{a}
200	9.51±0.42^{b}	10.5±0.54^{C}	66.23±3.04^{a}	78.9±1.4^{b}

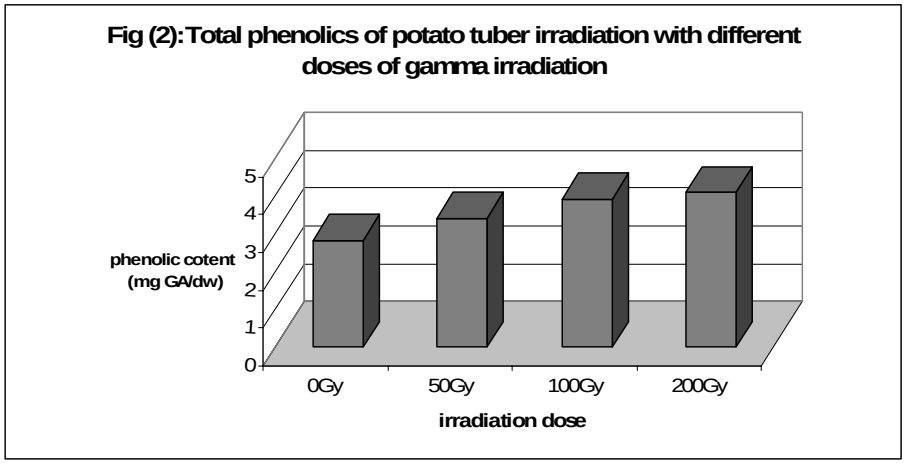
Vertically means bearing different letters are significantly different at P< 0.01.
*A.A : ascorbic acid ** b.wt = body weight

The above results lead to study the effect of irradiation on the potato tubers which might help in understanding the changes occurred to adult moths. Fig. (1) illustrates the effect of irradiation on vitamin C content of potato tubers. It is clear that irradiation at 100 and 200 Gy greatly reduced vitamin C content of potato tubers, i.e. it reduced an important nutritional competent.



A.A. Ascorbic acid

Due to the importance of secondary plant metabolites such as phenolics, the effect of irradiation on the phenolic content of potato tubers was estimated (Fig. 2). Irradiation significantly increased antinutritive or phenolic compounds at the highest and lowest doses. Phenolic contents were 2.81, 3.4, 3.9, 4.08 mg/g.b.wt for control, 50,100 and 200Gy irradiated potato tubers, respectively. This may indicates the sensitivity of the mechanism that involved in the formation of these compounds to gamma rays.



GA=Gallic acid, Dw=dry weight of the original plant sample.

The elevated phenolic level shown in Fig. (2) necessitated to study the effect of irradiation on oxidases of potato tubers as sign of the increased phenolic content. Table (3) shows that irradiation of potato tubers caused increasing in both peroxidases and phenoloxidases. As in phenolic content, oxidases gradually increased as the irradiation dose increased. We found that phenoloxidase increased more than peroxidase in potato tubers after irradiation, and the two enzymes significantly increased than in non-irradiated potato tubers. Peroxidase level of 200Gy irradiated potato tuber was about two folds as compared to control. It measured 91.3 and 50.17 O.D. unit x 10^3 /gm/min for 200Gy irradiated potato and control, respectively. On the other hand phenoloxidase activity at the same dose reached about five folds as compared to control potato tubers.

Table (3): Effect of gamma irradiation on the activities of peroxidase and phenoloxidase (means ± SE) of potato tubers.

Dose in Gy	Peroxidase * (O.D. unit x 10^3 /gm/min)	Phenoloxidase (O.D. unit x10^3/ min / gm. b.wt)
Control	50.17±2.25^{d}	0.39±0.06^{c}
50	67.5±2.1^{b}	1.125±0.76^{b}
100	84.4±2.16^{c}	1.21±0.08^{b}
200	91.3±1.4^{a}	1.59±0.1^{a}

Vertically means bearing different subscripts are significantly different at P< 0.01.
* 1 unit= 1 O.D. (optical density).

On the other hand, during preliminary experiments, it was observed that homogenates of the potato tuber moth adults fed as larvae on irradiated potato tubers, became dark faster than that of the adults fed as larvae on non-irradiated potato tubers. So oxidases of adults were studied due to their importance to insect immunity. Table(4) shows that feeding of larvae significantly increased humoral immune reaction of the adults. This is evident from the increased oxidases capacity in adult moths at most irradiation doses. However, peroxidases and phenoloxidases in females were not affected significantly affected at 50 Gy irradiation dose. Peroxidase activity was 93.1 and 92.7O.D. unit x 10^3 /min/ g.b.wt. for control females and females fed as larvae on 50Gy irradiated potato tubers, respectively.

Table (4): Activities of peroxidase and phenoloxidase enzymes (mean ± SE) of newly emerged females and males of *Ph. operculella* fed as larvae on irradiated potato tubers.

Dose in Gy	Peroxidase *(O.D. unit x 10^3 /min/ g.**b.wt)		Phenoloxidase (O.D. unit x10^3/ min / gm. b.wt)	
	Female	Male	Female	Male
0	93.1±2.7^{c}	155±2.5^{b}	2±0.1^{c}	3.1±0.1^{b}
50	92.7±1.5^{c}	169±2^{a}	2.2±0.1^{c}	2.9±0.1^{c}
100	143.3±4.2^{a}	170.2±3.7^{a}	3.2±0.1^{a}	3.9±0.1^{a}
200	125 ±2.7^{b}	144.3±8.4^{c}	2.8±01^{b}	3.3±0.1^{b}

Means bearing different subscripts are significantly different at P< 0.01.
* 1 unit= 1 O.D. (optical density) ** b.w= body wieght

Data were statistically analyzed by two ways ANOVA test to show the importance of both sexes and irradiation dose on the tested value pooled from adult moths of *Ph. operculella*. The data in Table (5), in general, clarified that both sexes and the dose were responsible on the significant change of the tested macromolecular abnormalities found in adult moths.

Table (5): Two ways ANOVA completely randomized indicting F ratio and P values and interaction of the effect of sex and irradiation dose on the insect.

Macromolecular components	Sex		Irradiation dose		Interaction (dose x sex)	
	F-ratio	P	F-ratio	P	F-ratio	P
Vitamin C	1.633	0.219	0.999	0.419	1.052	0.397
Peroxidase	796	0.000***	74.28	0.000***	70.21	0.000***
Phenoloxidase	460	0.000***	183.41	0.000***	18.77	0.000***
Alkaline phosphomonoestrases	635	0.000***	581	0.000***	124.06	0.000***
Acid phosphomonoestrases	776	0.000***	168	0.000***	171	0.000***
Trehalose	516	0.000***	72.28	0.000***	37.27	0.000***

***: F is significant at P< 0.01

DISCUSSION

The results obtained indicated that the change of larval diet due to irradiation led to some macromolecular abnormalities in the adult stage. This means that the effect resulting from larval feeding extends up to the mature stage. **Fernandes-Da-Silva and Zucoloto (1997)** found results which support the hypothesis that feeding the larval phase is more decisive for *Ceratitis capitata* in term of egg production during the oviposition phase than feeding during the adult phase.

Saour *et al.*, (1999) suggested that there are some nutritional factors (proteins, carbohydrates, vitamins or alkaloids) present in leaves and tubers of potato

plants that were grown from irradiated seeds (tubers) which could affect the larval developmental time and survival, fecundity and fertility of *Ph. operculella*.

The reported effects on the reproductive capacity of the tuber moth adults as a result of larval feeding on irradiated potato tubers, led to the study of some macromolecular components such as trehalose, vitamin C and phosphomonoesterases. Trehalose is the main source of energy in insects, and vitamin C or L-ascorbic acid has a vital importance for reproduction in insects **{Vanderzant and Richardson, (1963) and Moursy, (1995)},** besides phosphatases **(Rousell, 1971).** The present study illustrated that vitamin C content of the adults was not significantly changed by irradiation than control, while phosphatases activity and trehalose content were significantly depressed.

Although, in the present experiments, vitamin C reduced in the irradiated potato tubers as compared to non-irradiated tubers which agree with the findings of **Joshi *et al.,* (1990) and Fan *et al.,* (2003)**. The content of this vitamin in female moths was unchanged, while it slightly decreased in males at 50 and 200 Gy doses. From the nutritional point of view, this means that the decreases of a nutritional content in a diet not pertains a corresponding decrease in the fed insects. The insect may be compensate vitamins from symbionts **(Chapman, 1989),** or may increase the relative consumption rate. **Amin, (2008)** found that vitamin B deficiency in the diet of *Spodoptera littoralis* increased larval consumption of food. However, information on physiological behavior of *Ph. operculella* in not available and needs further investigation.

Haiba and Abd-El Aziz, (2008) reported significant decreases in proteins and carbohydrates of potato tubers, and both males and females of *Ph. operculella* at most doses (50-200 Gy). The reduction of total carbohydrates in the diet (potato tubers) after irradiation, could reasonably led to reduction of the adult main sugar; trehalose as observed in the present study.

The measured increase in phenolic content of potato tubers provide evidence that irradiation of potato tubers could leads to products which may be toxic to insects, besides their ability to affect nutritional requirements resulting in changes occur to the adult stage.

Irradiation increases phenolic content in a number of plants such as fresh cut vegetables **(Fan, 2005).** The increase in phenolic compounds was due to the increased phenylaniline ammonia lyase activity. Similar to mechanical wounding **(Kang and, Sltiveit 2002).** Irradiation can increase the activity of this enzyme resulting in the accumulation of phenolic compounds **(Tomas-Barberan and Espin, 2001).**

The direct toxicological impact of phenolic compounds, which considered as antinutritive compounds, on insects has been explored by authors such as **Summers and Felton, (1994).**

They reported that generation of reactive oxygen species due to conversion of phenolics to quninones may initiate oxidative the midgut epithelium of insects. If the oxidative stress imposed exceeds in the antioxidant resources of the midgut tissues, protein oxidation and lipid peroxidation should ensue. They added that biological consequences of oxidative stress may be impaired growth and development, and reduced reproductive potential.

Polyphenoloxidase catalyzes hydroxylation of monophenols to O-diphenols and dehydrogenation of O-Diphenol to O-quinone. Irradiation, in general, increase polyphenoloxidase and peroxidase capacity in fresh fruits and vegetables **(Tomas-Barberan and Espin, 2001).** Similarly in this study it was observed such increase in

phenoloxidase capacity not only in irradiated potato tubers, but also in adults of *Ph. operculella* fed as larvae on these tubers. Phenoloxidase activation is under moulting hormone control which is secreted by the prothoracic gland. The exact mechanism (s) for such observation needs further study. On the other hand, since activation of oxidases such as phenoloxidase and peroxidase considered as one of the important humoral immune responses of insects, it may suggest that irradiation of potatoes causes potato tuber moth to be less susceptible to microbial pesticides.

In conclusion, ionizing radiation not only inactivate food borne pathogens, improve hygienic quality of many fruits and vegetables, or directly kill stored product pests, but also could control reinfestation of insects by inducing changes in nutrients and increasing antinutritive compounds such as phenolics which might be toxic, and produce biochemical changes that may importance extend to the adult stage. This situation adds to the significant of irradiation.

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ARABIC SUMMERY

التغيرات البيوكيميائية في الطور الكامل لفراشة درنات الطاطس المغذاة في الطور اليرقي على درنات البطاطس المشعة

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تناولت هذه الدراسة أثر درنات البطاطس المعاملة بأشعة جاما (50، 100، 200جراي) على المكونات البيوكيميائية للطور البالغ لفراشة درنات البطاطس المغذاة في طور اليرقة على هذه الدرنات. وقد أظهرت النتائج حدوث بعض التغيرات في الطورالكامل نتيجة لذلك. فقد أدت عملية تشعيع الدرنات الى زيادة المحتوى الكلي للفينول وزيادة القدرة على الأكسدة في درنات البطاطس المشعة، بينما قلت كمية فيتامين سي في الدرنات. بالرغم من ذلك لم يتأثرمحتوى فيتامين سي في الطور البالغ للحشرة. وهذا يعني من وجهة النظر الغذائية أن نقص احد العناصرالغذائية في البطاطس ليس بالضرورة أن يؤدي الى نقصها فى اليرقات التي تغذت عليها. و لكن تأثر نشاط كل من الفوسفومنواستريز الحمضي و القاعدي و كذلك المحتوى الكلي للتريهلوز في الاطوار البالغة للفراشة. ويرجع هذا الانخفاض الى زيادة نسبة الفينول في درنات البطاطس المشعة. مما يعني ان تشعيع الدرنات يؤدي الى انتاج مواد قد تكون سامة للحشرات وقادرة على احداث تغير في المحتوى الغذائي في الدرنات التي تتغذى عليها اليرقات . و من ناحية أخرى أدت تغذية اليرقات على البطاطس المشعة الى تنشيط المناعة في الفرشات الكاملة. وربما يؤدي تغذية اليرقات على البطاطس المشعة الى قلة حساسيتها للمبيدات الميكروبية. و يتضح من هذه الدراسة أن تشعيع درنات البطاطس لا يؤدي فقط الى حمايتها من الاصابة بالامراض وقتل الحشرات الموجودة بداخلها ولكنه قد يؤدي الى التقليل من الاصابة بالافات الحشرية مرة اخرى نتيجة لاحداث تغير في القيمة الغذائية لدرنات البطاطس و زيادة المواد السامة و التي ربما تؤدي الى احداث تغيرات بيوكيميائية وتقليل القدرة على التكاثر في فراشة درنات البطاطس.