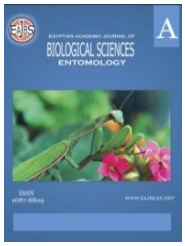
Egypt. Acad. J. Biolog. Sci., 13(2):205-214 (2020)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Effectiveness of Ozone Gas Against the Potato Tuber Moth (*Phthorimaea Operculella*) Infested Stored Potato Tubers

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ARTICLE INFO
Article History
Received:2/4/2020
Accepted:21/5/2020

Keywords:
Ozone - *Phthorimaea operculella* –potato tuber –sprouting-storage.

ABSTRACT

The effect of ozone against immature stages of the potato tuber moth (PTM) *Phthorimaea operculella* was investigated. The immatures (Eggs, the newly hatched larvae, 12days old larvae, and pupae) were exposed to ozone concentrations using two procedures. Thirty of isolated stages (first procedure of exposure) and 5 of infested potato tubers contain a separate stage of PTM (the second one) were exposed to ozone concentrations 50, 100, 200, 400, 600, 800 and 1000 ppm in a combination of different exposure times 2, 4, 6 and 8 hours. All results indicated that mortality percent of all insect stages increased by increasing the concentration and exposure time. The effect of ozone concentrations was higher on the isolated numbers of immature stages in the first procedure of exposure than that within potato tubers in the second one. The newly hatched larvae in both procedures were the most sensitive stage to ozone, it completely killed when exposed for 8hrs at 50 and 200 ppm in the first procedure and second procedure, respectively. While the elder larval stage was the least sensitive one. At the highest concentration and the longest exposure time (8hrs), larval mortality was 82.2 and 68.5 % in the two procedures, respectively. Also, sprouting of potato seeds was enhanced when treated with ozone concentrations compared with untreated seeds.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important source of nutrients and energy, globally representing the fourth most important food crop. In Egypt, potato production is increasing rapidly in response to increasing local and export demand for potato products. The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidotera, Gelechiidae) is one of the most important insect pests attacking potato crops in both field and storage. Females of moth lay eggs on both foliage and uncovered tubers by the soil, the larvae mine in the leaves, and infest the tubers (Rondon 2010**).** The fully-grown larvae crawl from the tubers to the soil and pupate in a cocoon (Trivedi *et al.* 1994; Das 1995; Saour 2004; Saour *et al.* 2012). Insecticidal formulations of *Bacillus thuringiensis* used in the potato tuber moth management, but with limited success (Kroschel and Koch 1996; Arthurs *et al.* 2008; Kumar *et al*. 2010).

Ozone (O3) is triatomic of oxygen and is referred to as activated oxygen, or allotropic

oxygen. It is an unstable gas with a half-life of about 20 min depending on the temperature. Thus, it does not persist and therefore accumulates substantially without continual generation (Peleg, 1976; Miller et al., 1978). Also, ozone gas is an excellent alternative to current methods, ozone has the ability to sanitize, disinfect, and is generally recognized as safe for food processing in the United States (Sopher et al., 2002; Campabadal et al. 2013). (Alencar et al., 2014) studied the effect of ozone (O3) of 100 ppm for 60 minutes on pear's quality. Results of treated pears quality showed that the microorganism counts in ozonated pears were lower than those in untreated pears and ozone increased the shelf life of the pears with efficient microorganism control.

Many investigators studied the efficacy of ozone against stored grain and product insects (Marissa .*et al*., 2011; Ensieh *et al.* 2014; Exinyi and Beibei, 2017).

Ozone application did not significantly or systematically alter the sprouting of seed potatoes. There was a slight increase in the sprouting rate, particularly at high ozone concentrations. Any acceleration of sprouting could be a stress response reflecting the accelerated aging of the potatoes by exposure to ozone. Aging or other stresses break tuber dormancy in potatoes (Burton, 1966). Daniels-Lake *et al.* 1996, found that ozone treatments (up to 100 ppm) did not reduce sprouting or visible tuber quality in stored potatoes. The aims of this study to evaluate the efficacy of ozone against different stages of the potato tuber moth (PTM) *Phthorimaea operculella* and potato seeds sprouting.

MATERIALS AND METHODS

Insects:

The stock culture of potato tuber moth (*Phthorimea operculella* (Zeller) reared at Stored Grain &Product Pests Res. Dept., Plant Protection Research Institute (PPRI), ARC, Dokki, Egypt. Twenty pairs of newly emerged moths were collected and entered in 800 ml transparent plastic jars contain washed and sterilized potato tubers. The jars were covered with a muslin cloth and fixed with a rubber band then incubated under laboratory condition at 26 ±1°C, 70% RH, and 16:8 (L:D) photoperiod. This procedure was repeated several times in order to obtain larger numbers of the adults needed to carry out the experiments. The food in the jars was renewed when it was necessary.

Obtaining of the Insect Developmental Stages:

1- In the First Technique of Exposure:

1.1 Egg Isolation:

Five couples of moth adults were put in a glass cage with gauze lid (mesh width 2 mm) and a piece of black paper above the gauze. The cage was covered with a muslin cloth and fixed with a rubber band; Eggs found on this paper were collected daily.

1.2 The Newly Hatched Larvae:

Eggs on the paper were entered into a petri dish and investigated after 3days to collect the newly hatched larvae.

1.3 The Elder Larvae and Pupae:

The elder larvae (12days old) and pupae for tests were taken from the media.

2. In the Second Technique of Exposure:

At first, biological tests were performed in order to determine the duration of the various developmental stages at rearing conditions. 100 couples of newly emerged moth were introduced to small potato tubers in plastic jars for 24hrs then separated from the jars. Five of the infested potato tubers within the lonely stage of the egg (24hrs old), the newly larvae (24hrs old), larvae (12days-old), and pupae (24-48hrs old) were taken from the prepared culture jars.

3. Ozone Generator:

Ozone gas was produced from the air using an ozone generator Model OZO 6 VITL OZO Max Ltd, Shefford, Quebec, Canada (OZO Max Ltd, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the laboratory of Food Toxicology and Contaminants, National Research Center. Ozone generator provided by a monitor- controller having a plug-in sensor on board which allows controlling the concentration in a selected range Fig.(1).

Fig.(1) Ozone apparatus

4. Ozone Exposure Procedures:

Separate immature stages of *P. operculella* (Egg, the newly hatched larva, 12days old larva and pupa) were exposed to ozone at concentrations 50, 100, 200, 400, 600, 800 and1000 ppm for various exposure times 2, 4, 6 and 8 hours (hrs). The immatures were exposed to ozone concentrations using two procedures. Thirty of the isolated stage (first procedure of exposure) and 5 of infested potato tubers contain a separate stage of PTM (the second one). The prepared stages were introduced to small jute bags. Bags were closed well and secure with thread bands. Three replicates for each treatment or untreated were used. All bags were inserted inside a glass container (4 liters capacity each), closed tightly with a rubber stopper with 2 holes; one hole for the ozone line, and the other hole for tubing connected to the ozone destruct unit. At the end of the exposure time, the fumigation chamber was opened. Replicates of all treatments and control were taken and transferred to the laboratory for mortality assessment. The larval mortality was assessed after 24 hrs from aeration. Replicates of eggs and pupae in the first procedure and immature stages within the infested potato tubers in the second procedure poured into a plastic box, then covered and incubated in experimental conditions. Boxes were daily investigated for 9 days to count the hatched larvae and emerged adults from pupae. Mortality percent was assessed as the reduction in egg hatchability or emergency % compared with untreated (control) using the following equation:

Reduction % = $N_0 - N_1 / N_0 * 100$
N0 = No. of hatched larvae or adults emerged in control.
N_1 = No. of hatched larvae or adults emerged in treatment.

5. Effect of Ozone on Sprouting in Potato Seeds:

Potato seeds were obtained from the Potato brown rot project (Central Administration of Seed Certification), 5 whole de-sprouted Spunta seeds were exposed to different concentrations of ozone for 2hrs. All replicates poured into a plastic box after the end of the exposure, then covered and incubated in rearing conditions. The number of eyes was counted and the number of eyes that had sprouted was determined for each tuber after 4, 7 and 14 days of storage, and the final sprouting percentage was calculated.

6. Data Analysis:

The lethal concentrations of ozone to the different stages of *P. operculella* after 8hrs of exposure were statistically analyzed according to Finney (1971).

RESULTS AND DISCUSSION

The response of various immature stages of PTM in the two procedures of exposure to ozone concentrations for different exposure time is shown in Tables (1&2).

1. In the First Procedure:

Results of the isolated immature stages of PTM presented in Table 1.

1.1. Eggs:

The mortality percentages of a specific number of PTM were increased gradually with an increase in the concentration of ozone and exposure time. After 2 hrs of exposure at the lowest concentration (50 ppm), the mortality percentage was 40.0 % and increased gradually to reach 76.7% when the concentration of ozone increased to 1000 ppm. Meanwhile, the corresponding values after 8 hrs of exposure were 47.8 and 85.6 % at 50 and 1000 ppm concentrations, respectively.

1.2. The Newly Hatched Larvae:

The highest effect of ozone was achieved against the newly hatched larvae, the lowest concentration (50 ppm) caused high mortality percentages 96.7, 97.8, and 98.9% after 2, 4 and 6 hrs of exposure, respectively. Also, complete kill of the newly hatched larvae was obtained after 8hrs of exposure at 50 ppm.

1.3. The Elder Larvae:

The elder larvae of PTM (12days- old) was more tolerant to ozone, mortality percentages were 35.6, 48.9, 52.2, 61.1, 63.3, 73.3 and 75. 6 % after 2hrs of exposure at 50, 100, 200, 400, 600, 800 and 1000 ppm, respectively. These values were 46.7, 56.7, 66.7, 72.2, 73.3, 82.2, and 84.4 % when the time of exposure was prolonged to 8 hrs at the aforementioned concentrations, respectively.

1.4. Pupae:

The effect of ozone on pupae was higher than eggs and the elder larvae, mortality percentages of the pupae were 46.7, 54.4, 67.8, 73.3, 75.6, 78.9, and 80.0% after 2hrs of exposure at the tested concentrations, respectively. The corresponding values after 8hrs of exposure were 58.9, 66.7, 81.1, 84.4, 88.9, 90.0, and 91.1% at the above-mentioned concentrations, respectively.

2. In the Second Procedure:

The effect of ozone concentrations and exposure time on *phthorimaea operculella* immature stages within the infested potato tubers are summarized in table 2.

2.1. Eggs:

The effect of ozone concentrations was low on the eggs laid on the infested potato tubers, the egg hatchability was reduced by 31.6 and 62.0% after 2 hrs of exposure at 50 and

1000ppm, respectively. The corresponding values after 8 hrs of exposure were 40.2 and 69.2 % at the above-mentioned concentrations, respectively.

2.2. The Newly Hatched Larvae:

The effect of ozone against the newly hatched larvae within the infested potato tubers was high, but it was lower than that obtained against the isolated larvae in the first procedure, the newly hatched larvae completely killed after exposure to ozone at 200 ppm for 8hrs, while only 2hrs of exposure at 400 ppm was sufficient to obtain a similar effect.

2.3. The Elder Larvae:

The elder larvae of PTM within the infested potato tubers was the least sensitive stage to ozone, at the highest concentration (1000 ppm) and the longest exposure time (8 hrs), the mortality percentage was 68.5 %, while the corresponding value at 50 ppm was only 32.2%.

Table 1: The effect of ozone concentrations and exposure time on isolated immature stages of *phthorimaea operculella*

Conc. (ppm)	Stage	Mortality% ±S.E. after indicated Exposure time(hours)			
		2	4	6	8
50	Egg	40.0±1.9	41.1±2.9	46.7±0.0	47.8±2.9
	N. larva	96.7±1.9	97.8±1.1	98.9±1.1	100.0±0.0
	E. larva	35.6±2.9	40.0±1.9	42.2±1.1	46.7±1.1
	Pupa	46.7±3.3	47.8±1.1	53.3±1.9	58.9±1.1
100	Egg	54.4±2.2	56.7±3.3	60.0±1.9	62.2±1.1
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	48.9±2.9	50.0±3.8	52.2±1.1	56.7±0.0
	Pupa	54.4±1.1	60.0±1.9	64.4±2.9	66.7±0.0
200	Egg	57.8±2.2	60.0±1.9	65.6±0.7	71.1±1.1
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	52.2±2.9	53.3±1.9	58.9±2.2	66.7±3.8
	Pupa	67.8±2.9	71.1±2.2	76.7±1.9	81.1±2.2
400	Egg	63.3±1.9	68.9±1.1	72.2±1.1	73.3±1.9
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	61.1±2.9	65.6±1.1	70.0±1.9	72.2±2.2
	Pupa	73.3±3.3	76.7±0.0	80.0±1.9	84.4±2.9
600	Egg	66.7±3.3	71.1±1.1	73.3±1.9	77.8±2.9
	N. larva	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
	E. larva	63.3±1.9	71.1±1.1	72.2±2.9	73.3±3.8
	Pupa	75.6±2.9	80.0±1.9	85.6±2.2	88.9±1.1
800	Egg	74.4±1.1	77.8±2.2	81.1±1.1	83.3±1.9
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	73.3±1.9	75.6±2.2	78.9±1.1	82.2±2.9
	Pupa	78.9±1.1	81.1±2.9	85.6±2.2	90.0±1.9
1000	Egg	76.7±1.9	80.0±1.9	82.2±1.1	85.6±2.2
	N. larva	100.0±0.00	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	75.6±2.2	77.8±2.9	80.0±1.9	84.4±2.2
	Pupa	80.0±1.9	82.2±2.2	86.7±1.9	91.1±1.1

N. larva = The newly larva, E. larva = The elder larva, S.E. = Standard error

Table 2: The effect of ozone concentrations and exposure time on immature stages of *phthorimaea operculella* within infested potato tubers

Conc. (ppm)	Stage	Mortality % ± S.E. after indicated exposure time (hours)			
		2	4	6	8
50	Egg	31.6±0.9	36.7±1.1	39.1±1.0	40.2±0.6
	N. larva	37.8±0.8	40.0±1.1	44.4±1.0	46.9±1.4
	E. larva	25.7±1.0	28.9±1.4	30.0±0.5	32.2±1.2
	Pupa	34.4±1.3	38.9±1. 4	40.3±1.3	45.5±1.2
100	Egg	33.2±1.8	36.8±1.3	43.1±1.2	45.3±1.1
	N. larva	52.2±1.2	61.9±1.3	64.4±1.0	65.8±1.5
	E. larva	27.8±1.0	30.5±0.8	31.2±1.6	39.7±1.4
	Pupa	41.0±0.9	43.2±1.5	45.5±1.2	49.3±0.9
200	Egg	41.2±1.3	48.9±1.1	53.5±1.0	55.8±1.1
	N. larva	89.7±1.5	91.1±1.3	95.7±1.2	100.0±0.0
	E. larva	31.1±0.8	33.2±1.8	36.5±1.1	46.1±1.5
	Pupa	46.5±0.9	55.1±1.4	56.8±1.0	58.9±1.2
400	Egg	46.8±0.9	52.4±1.0	57.4±1.1	60.3±0.9
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	41.1±1.0	50.3±1.2	53.5±0.9	56.8±1.0
	Pupa	52.3±1.3	56.8±0.9	61.2±1.0	63.1±1.2
600	Egg	52.1±1.6	58.4±0.9	63.1±1.3	66.0±1.1
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	51.4±1.3	56.1±1.4	62.3±0.9	64.6±1.2
	Pupa	57.9±1.1	63.7±0.9	66.7±0.9	69.0±1.0
800	Egg	61.4±1.2	63.2±0.9	65.9±1.0	68.9±1.6
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	60.4±1.0	62.0±0.9	65.6±0.8	68.1±1.2
	Pupa	62.3±0.8	64.5±1.0	67.9±1.2	72.0±0.6
1000	Egg	62.0±1.2	64.5±0.8	66.6±1.4	69.2±1.2
	N. larva	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
	E. larva	61.5±1.0	63.3±0.8	66.4±0.9	68.5±1.4
	Pupa	63.1±1.3	65.0±0.9	68.7±1.1	73.2±0.8

N. larva = The newly larva, E. larva = The elder larva, S.E. = Standard error

2.4. Pupae:

The mortalities of pupae within potato tubers were less than 50% when exposed to ozone at the lowest concentrations 50 and 100ppm for the longest time of exposure (8 hrs). The mortality percentages were increased with increasing of concentration to reach at 1000 ppm 63.1, 65.0, 68.7and 73. 2% after 2, 4, 6, and 8hrs of exposure, respectively.

3- Lethal Concentrations of Ozone to The Immature Stages of *Phthorimaea Operculella* After 8hrs Of Exposure:

The calculated lethal concentrations of ozone to the immature stages of *phthorimaea operculella* after 8hrs of exposure to ozone concentrations are summarized in Table 3. In the case of isolated stages (first procedure), the mean values of LC_{50} and LC_{90} of egg, elder larva and pupa were 50.3, 74.5 and 29.1; 215.1, 2808.3 and 778.3 ppm, respectively. The same trend could be applied for egg, larva and pupa within potato tubers (second procedure). The results of mortality percentages for the newly larvae exposed to ozone concentrations for tested exposure time and different stages of the tested insect exposed to ozone concentrations

for 2, 4 and 6 hrs not subjected to statistical analysis.

Results indicated that mortality percent for immature stages increased by increasing the concentration and exposure time. Also, the effect of ozone concentrations was higher on the isolated immature stages than those within potato tubers. The newly hatched larvae were the most sensitive stage to ozone in both procedures. While the elder larval stage was the least sensitive one. The eggs and pupae of potato tuber moth were more sensitive to the modified atmosphere than larvae and adults, (Ibrahim and Al-Ahmadi, 2014**)**. (Hansen *et al.* 2012) tested the effect of continuous flows of ozone in doses of 10–135 ppm and exposure times of 5–8 days against the freely exposed and internal stages of eleven stored-product pest species. Test insects were three species of *Sitophilus*, *R. dominica*, *Tribolium confusum* Jacquelin du Val, *T. castaneum* Herbst, *Plodia interpunctella* H ubner, *Sitotroga cerealella* Olivier, *Oryzaephilus surinamensis* L., *Ephestia kuehniella* Zeller and *Stegobium paniceum* L. results revealed that the freely exposed stages of the eleven species were more susceptible to ozone compared to the internal stages.

Table 3: LC_{50} and LC_{90} values with their confidence limits for immature stages of *phthorimaea operculella* after 8hrs of exposed to ozone concentrations.

Stage	Lethal concentrations (ppm) and their 95% confidence limits		Slope ± S.E	R
	LC_{50}	LC_{90}		
(Procedure No.1) Isolated stages				
Egg	50.3 (23.8 - 81.3)	215.1 (1232.9 - 5647.4)	0.79 ± 0.12	0.98
E. larva	74.5 (40.7-109.6)	2808.3 (1564.7-7614.4)	0.81± 0.12	0.98
Pupa	29.1 (12.1-49.1)	778.3 (531.6-1407.9)	0.90 ± 0.13	0.99
(Procedure No.2) Stages within potato tubers				
Egg	134.4 (74.5 - 197.0)	174306.6 (5777.3 - 16239809.2)	0.61 ± 0.11	0.99
E. larva	220.9 (158.4-293.8)	10313.4 (4568.6-42224.9)	0.77 ± 0.11	0.99
Pupa	89.5 (40.7-109.6)	13776.9 (4669.4-130412.3)	0.59 ± 0.13	0.99

R = Correlation coefficient of regression line.
S.E = Standard error of regression line.

4. Effect Of Ozone On Potato Seeds Sprouting:

Results of the influence of ozone concentrations on potato seeds sprouting are presented in table 4. The observed data at 4 days of the investigation indicated that the mean percentages of sprouts in potato seeds treated with all ozone concentrations were enhanced compared with untreated seeds. At 14 days of investigation, potatoes exposed to the two lowest ozone concentrations (50 and 100ppm) for 2hrs had the lowest percentages of sprouts (91.9 and 92.9 %-) compared with untreated potatoes which had (95.4%). While the potatoes sprouting was increased by increasing ozone concentrations and recorded (99.1 and 99.0%) at (200 and 400ppm). Potatoes exposed to 80 or 320 mg O3/kg/hr had a significantly higher

percentage of their eyes with obvious sprouts than did the control or 20 mg O3/kg/hr treatments after four days after treatment**,** Robert (2003).

The obtained results, ozone appears to have a moderate potential as PTM management agent in stored potatoes in an integrated management program but, it requires more studies to increase the efficacy of ozone by using much higher dosages over a very short time just as potatoes are coming into storage.

Table 4: Effect of ozone concentrations on of potato seeds sprouting

Conc. (ppm)	Sprouting % ± S.E. at indicated periods(days)		
	4	7	14
50	51.4±1.3	82.4±1.0	91.9±0.7
100	64.1±1.2	83.1±1.3	92.9±1.4
200	52.0±1.0	84.4±0.6	99.1±0.5
400	68.7±2.6	82.6±2.0	99.0±0.5
600	70.7±1.8	84.2±0.7	97.7±0.6
800	69.1±2.1	80.9±0.3	96.0±0.6
1000	77.1±1.5	81.6±0.5	95.9±0.9
Control	47.1±1.2	82.9±1.1	95.4±1.3

Acknowledgements

The authors are grateful to the potato brown rot project (Central Administration of Seed Certification) for providing potato seeds.

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ARABIC SUMMARY

فاعلية غاز الأوزون ضد فراشة درنات البطاطس (*Phthorimaea operculella*)
التى تصيب درنات البطاطس المخزونة

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تم دراسة تأثير غاز الأوزون ضد الأطوار الغير كاملة لفراشة درنات البطاطس(PTM) *Phthorimaea operculella*. تم تعريض الأطوار الغير كاملة (البيض، اليرقات حديثة القفس، اليرقات عمر ١٢ يومًا و العذارى) لتركيزات الأوزون بطريقتين. تم تعريض عدد ثلاثون من كل طور منفرداً (في الطريقة الأولى) وتم تعريض ٥ درنات بطاطس مصابة بها أحد الأطوار لفراشة PTM (في الطريقة الثانية) لتركيزات من الأوزون ٥٠، ١٠٠، ٢٠٠، ٤٠٠، ٦٠٠ ، ٨٠٠ ، ١٠٠٠ جزء في المليون ولفترات تعريض مختلفة ٤،٢، ٦، ٨ ساعات.

وقد أوضحت النتائج أن نسبة الموت لجميع الأطوار قد إزدادت بزيادة التركيز وفترات التعريض. وكان تأثير تركيزات الأوزون على أعداد الأطوارالغير كاملة فى الطريقة الأولى للتعريض أعلى منه فى الأطوار داخل درنات البطاطس المصابة فى الطريقة الثانية للتعريض. وكانت اليرقات حديثة الفقس أكثر حساسية للأوزون في كلا من طريقتى التعريض، حيث قتلت تمامًا عندما تعرضت لمدة ٨ ساعات على تركيزى ٥٠، ٢٠٠ جزء في المليون في الطريقة الأولى والثانية على التوالي.

بينما كانت مرحلة اليرقات الاكبر عمراً أقل حساسية. حيث كانت نسب الموت اليرقات عند أعلى نسبة تركيز وأطول فترة تعرض (٨ ساعات) هى ٨٢,٢٪ و ٦٨,٥٪ في الطريقة الأولى والثانية على التوالي.

كماوجد ايضاً أن نسبة التبرعم لتقاوى البطاطس قد تحسنت بعد تعريضها لتركيزات من الأوزون مقارنة بالتقاوى غير المعاملة.