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Effect of nano-silica extracted from two different plant sources on survival and development of *Phthorimaea operculella* (Zeller) larvae

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Summary The study examined the effect of nano-silica extracted from two different plant sources on the survival and development of the potato tuber moth, *Phthorimaea operculella*. The silica powder was derived from two different agricultural byproducts, olive stones and corncobs. Characterization by X-ray diffraction revealed that the extracted powder has an amorphous silica phase. The nitrogen adsorption-desorption measurements revealed that both extracted and treated silica have mesoporous structure, with a specific surface area of around 300 m^2/g and 270 m^2/g for the silica derived from olive stones and corncobs, respectively. The silica nanoparticles (SiO_2 NPs) prepared from the silica derived from olive stones showed higher larvae mortality, pupae weight, and larval and pupal developmental time, compared to the silica derived from corncombs. The results show that the nano-silica derived from agriculture byproducts can be as effective as the synthetic insecticide (deltamethrin) utilized in control of the potato tuber moth, with lower environmental impact in terms of preventing pesticide residue accumulation. In addition, the efficiency of SiO_2 NPs applications depends on the source of the silica nanoparticles and the applied concentration to achieve the optimum results for the pest control.

Additional keywords: BET, corncobs, olive, potato tuber moth, SiO_2 NPs, XRD

Introduction

Potato (*Solanum tuberosum* L.) is the second most widely cultivated edible crop of Syria, after wheat (*Triticum aestivum* L.); the annual cultivation area is 22 thousand hectares and the production is 562 thousand tons of potato. Furthermore, potato is one of the most important economic crops in the southern and central regions of Syria (e.g., mainly in Damascus suburbs and Homs provinces) (Statistical data, 2019). Indeed, potato crop has been identified as a key element for ensuring national food security and reducing poverty levels (Zhang *et al*., 2021).

The potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelatiidae), can damage potato crop by 50% to 100% in tropical and subtropical regions of America, Africa, Europe, Oceania, Australia and Asia (Clough *et al*., 2010). The pest causes about 20-30% infestation in the field while the infestation can reach 100% during the storage period (Ahmed *et al*., 2016; Idris and Shoaib, 2019), thus it can seriously disturb the success of potato as a commercial crop.

Phthorimaea operculella larvae primarily infest potato by feeding on leaves, stems and petioles, and by making tunnels in potato tubers, causing damage to the crop (Chandel *et al*., 2010; Zhang *et al*., 2021). Pesticide applications are the dominant method for the control of the pest. However, due to their non-selectivity properties, resistance development and environmental issues (Idris and Hussian, 2021), there in an increased interest by the global potato industry to find effective alternative methods to control *P. operculella* (Harba and Idris, 2018; Zhang *et al*., 2021).

The technique of nanoparticles supports more environmentally friendly pesticides (Papanikolaou *et al*., 2018; Wang *et*

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al., 2022). Compared with nanoparticles used in other fields, agricultural nanoparticles, such as TiO_2, Al_2O_3 and SiO_2 nanoparticles (NPs), are generally much more affordable and easier to produce than traditional nanoparticles (Biswal *et al.,* 2011; El-Bendary and El-Helaly, 2013; El-Helaly *et al*., 2016). Silica, silicon dioxide, nanoparticles (SiO_2, NPs) are increasingly used in plant treatments in the field of plant protection. They have shown great potential when they are applied appropriately in agriculture. Researchers report that the development of nanotechnology has increased the efficiency of chemical and organic pesticides, e. g. nanosilica has been found to be effective against the polyphagous plant pest *Spodoptera littoralis* (Lepidoptera: Noctuidae) (El-Bendary and El-Helaly, 2013). Moreover, nano-silica can reduce biotic and chemical stress on plants, such as salt, metal toxicity, and nutrition deficiency (Hersanti *et al*., 2018). Due to its simplicity, efficiency and low cost, the extraction of silica from plants and agriculture byproducts is increasingly used as a competent method for preparation of SiO_2 NPs (Goswami and Mathur, 2022, Naddaf *et al*., 2020, Mor *et al*., 2017). However, obtaining methods of the silica has been found to affect the SiO_2, NPs efficiency for agricultural applications (Karunakaran *et al*., 2013).

The purpose of this study is to: a) characterize the microstructure properties of silica powder extracted from olive stones and corncobs, by X-ray diffraction (XRD); b) determine the specific surface area and total pore volume of silica by measuring the nitrogen absorption-desorption; c) investigate whether the produced silica nanoparticles affect the life course of *P. opercullela* larvae including larvae mortality, pupae weight, and development time of larvae and pupae.

Materials and Methods

Insects rearing

Phtorimea operculella larvae were obtained from our laboratory stock cultures. They were reared on wax coated potato slices, maintained at a constant temperature of 25 ± 1°C, with 70 ± 5% relative humidity, and 12-hour light/darkness cycle as described by Makee and Saour (2003).

Silica preparation and characterization

Silica powder was extracted from corncobs and olive stones by the alkali leaching extraction method which is described in details in the study by Naddaf *et al*. (2020). The microstructure properties of the extracted silica powder were characterized by x-ray diffraction (XRD), using a STOE Powder diffractometer. Nitrogen adsorption-desorption by the Brunauer-Emmett-Teller (BET) method was used to determine the specific surface area and the pore size distribution of the studied samples. The measurements were performed at 77 K using Quantachrome NOVA 2200 BET surface area analyzer. The sample was vacuum-outgassed before the measurements for 12 hours at 150°C. The nitrogen adsorption-desorption isotherms were carried out in the range of relative pressure [P/P_0] from 0.05 to 1 atm.

Application of SiO_2 NPs on *P. operculella*

SiO_2 NPs were prepared by milling the extracted silica powder in a porcelain mortar, dispersing it in double distilled water (1.5 mg/ml), and ultrasonication for 30 min at a frequency of 30 kHz using an ultrasonic homogenizer with a tip in order to break big cluster agglomerates. By diluting the stock solution (1500 ppm), two more concentrations of SiO_2 NPs were prepared: 1000 and 500 ppm.

Potato tubers of the cv. "Draja" were planted in 10 L plastic (N=8 pots) containers containing moistened soil (three tubers/pot). Plants were grown in greenhouses at 25°C during the day and 23°C at night with daylight of 16 hours and a relative humidity of 85-95%. All plants were watered and fertilized the same way. Groups of eight fresh leaves were excised randomly from potato plants (six to seven weeks old) and sprayed with three concentrations (1500 ppm, 1000 ppm, 500 ppm) of amorphous olive nanoparticles and amorphous corncobs nano-

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particles. A pyrethroid insecticide (deltamethrin) was used as a positive control and sterile water as a negative control.

A total of 200 potato tuber moth larvae (24h old) were fed on leaves in 10 plastic boxes (18 x 12 x 8 cm) for each treatment (20 larvae per box). The boxes were sealed with parafilm to prevent larvae from escaping until they reached the pupa stage. Incubation took place at 25 ± 1˚C with daylight 12h and relative humidity of 70%. Development time was recorded for 30 pupae and larvae in each treatment. The number of emerging pupae was recorded and the percentage of larval mortality was calculated: Percentage of mortality = (number of larvae tested - number of pupae emerging) / number of larvae tested x 100. The experiment was repeated three times. Twenty five pupae per treatment (four days old) were randomly selected and weighed.

Statistical analysis

The statistical analysis was done using the STATISTIC program version 6 (Statsoft, Inc. 2003) at 5% significance level (p=0.05). Analysis of variance was performed on the data of mortality and pupae weight and Tukey HSD test was used for the separation of means.

Results and discussion

Characterization of SiO_2 NPs by XRD technique

The obtained silica from corncobs shows XRD pattern similar to the reported XRD pattern for the olive stones silica (Naddaf *et al.*, 2020) (Fig. 1). Figure 1 shows very broad peak centered at 2θ=20.26°, which corresponds to typical features of amorphous silica (Mor *et al.,* 2017). The XRD results were assisted by nitrogen adsorption-desorption measurements using the BET method. Figure 2 shows the nitrogen adsorption-desorption isotherm of the studied silica samples. Based on the six types of isotherm proposed by the International Union of Pure and Applied Chemistry (IUPAC) for classification of porous materials (ALOthman, 2012), the observed isotherms in our case exhibit characteristics of IV type which corresponds to a mesoporous material. Moreover, the specific surface areas of the studied samples were determined as follows: The silica derived from olive stones has a specific surface area of 300 m^2/g and a total pore volume of 0.327 cm^3/g. The corresponding values for the silica from corncobs were 270 m^2/g and 0.678 cm^3/g, respectively. The inset in Figure 2 shows the pore size distribution of the studied silica samples obtained from the nitrogen adsorption-desorption measurements by the Barrett-Joyner-Halenda (BJH) method. Both samples show pore diameter distribution that consists of mesoporous materials. However, the sample extracted from olive stones shows a maximum pore value of ~ 3.7 nm, which is more than three times lower than the maximum pore value (~ 12.4 nm) of the sample extracted from corncobs. This shows that the extraction source has an important impact on both porosity and particle size of the produced silica. This is in agreement with reported results by Karunakaran *et al*. (2013).

Effect of SiO_2 NPs on mortality and development of *P. operculella*

Mortality of the silica-treated *P. operculella* larvae at 1500 ppm was similar (corncob)

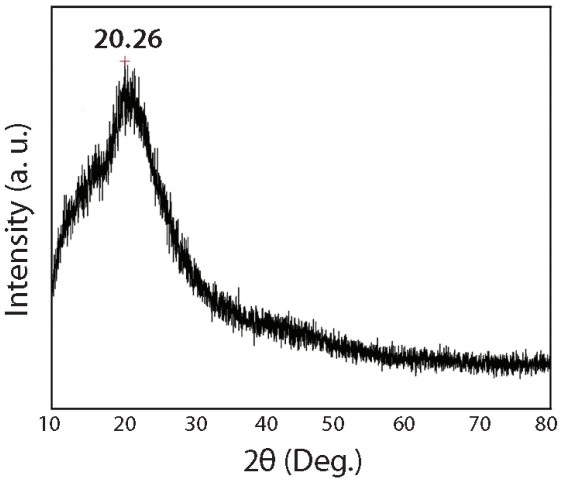


Figure 1. XRD of the extracted silica powder from corncobs, the very broad peak centered at 2θ=20.26° indicates the amorphous nature of the extracted silica.

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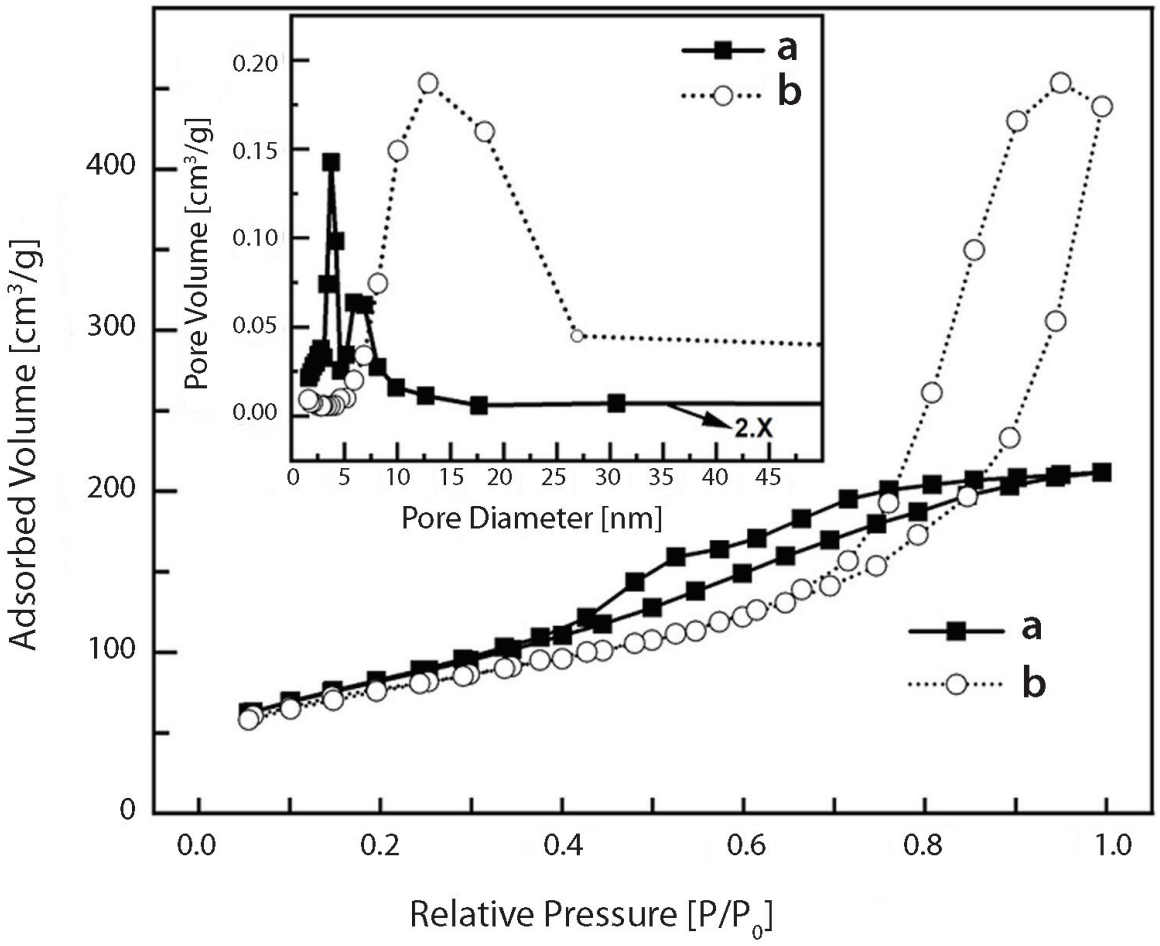


Figure 2. Nitrogen adsorption-desorption isotherm of silica samples extracted from: (a) olive stones and (b) corncobs; the corresponding pore distribution of each sample is depicted in the inset of the figure.

or statistically higher (olive stone) compared to that in deltamethrin treatment whereas larval mortality in the untreated control was significantly low (Fig. 3). Larval mortality in corncob SiO_2 NPs-treated larvae at the concentration of 1500 ppm did not statistically differ with that of the pesticide -treated larvae (DF=7, F-value=63.82, p<0.0001). The mortality of olive SiO_2 NPs-treated larvae was higher than the mortality of the corncob SiO_2 NPs -treated larvae. The effectiveness of silica nanoparticles in controlling insect pests has been demonstrated by numerous studies, and these results corroborate those findings. Previous studies on three mosquito species show a significantly higher mortality rate for larvae and pupae following exposure to SINPs (Cáceres *et al*., 2019). Usha *et al*. (2014) showed that terpene compounds significantly increased the toxicity of SiO_2 NPs in two lepidopterous pest species when combined with chemical compounds. The silica gel formulation with essential oils was also used to control two grain storage beetles, *Tribolium confusum* and *Sarocladium oryzae,* and resulted in high mortality rates for both species, which lasted for 7, 14 and 21 days after exposure (Athanassiou *et al*., 2013).

The amorphous phase of silica powder can cause more damage to larvae's midgut epithelium due to the mechanism of action leading to the insect's death (Mommaerts *et al*., 2012; Santo-Orihuela *et al*., 2016). Nevertheless, it is possible that the amorphous nanoparticles from olive-stones caused a higher mortality rate for larvae because the particles were smaller than corncob nanoparticles (Fig.1). In addition, silica obtained from olive stones has a higher specific sur-

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face area, and lower total pore volume (300 m^2/g, 0.327 cm^3/g) than silica obtained from corncobs (270 m^2/g, 0.678 cm^3/g). In an assessment of nanoparticle nanoformulations, researchers found that nanoparticle toxicity was improved in smaller size (Debnath *et al*., 2011). On the other hand, Santo-Orihuela *et al*. (2016) detected significant differences in the concentration of nanoparticles in *Spodoptera frugiperda* Sf9 cells as a size-dependent effect of nanoparticles.

Pupae in SiO_2 NPs treatments had statistically lower weight when compared with the control pupae. The lowest weight was observed in olive SiO_2 NPs treatment at 1500 ppm (DF=7, F-value=15.23, p<0.0001) (Fig. 4). Studies on the effect of silica nanoparticles on insects report cuticular damage, desiccation (Benelli *et al*., 2018; Cáceres *et al*., 2019), cuticle abrasion and spiracle obstruction (Shoaib *et al*., 2018). These factors could possibly contribute to the mortality and the reduction of pupae weight as a result of physiological disruption caused by SiO_2 NPs.

Larval development time differed between the treatments: it was statistically longer in the olive nanoparticles (1500 ppm, 1000 ppm) and corncobs (1500 ppm) treatments compared to other treatments and the control (DF=7, F-value=12.976, p<0.0001) (Table 1). The reason for the prolonged time of larval development could be attributed to the difficulties that larvae experience in feeding due to the nanoparticle treatment. As a result, larvae at high nanoparticle concentrations require longer time to reach the final instar and to pupate. This is in agreement with previous studies showing that larvae treated with toxic agents have longer development time (Adam *et al*., 2016; Idris *et al*., 2019; Idris and Hussian, 2021). Additionally, pupation time differed between treatments (DF=7, F-value=9.23, p<0.0001). The longest pupation time was recorded under the treatment with SiO_2 NPs of olive stone, at a concentration of 1500 ppm, compared to the other treatments and the control. According to El-Bendary *et al*. (2013), when larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) ingested nanoparticles of silica, the pupal period was extended in comparison with the control.

Conclusions

Nanoparticles of amorphous silica powders extracted from olive stones and corncobs are effective against *P. operculella,* with

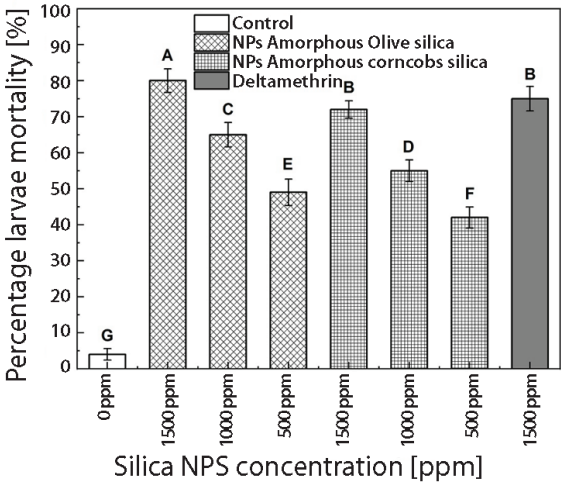


Figure 3. Effect of SiO_2 nanoparticles, derived from corncobs and olive stones, on larvae mortality of *Phthorimea operculella.* Different capital letters on top of columns indicate significantly different means at p<0.05 (Tukey HSD test).

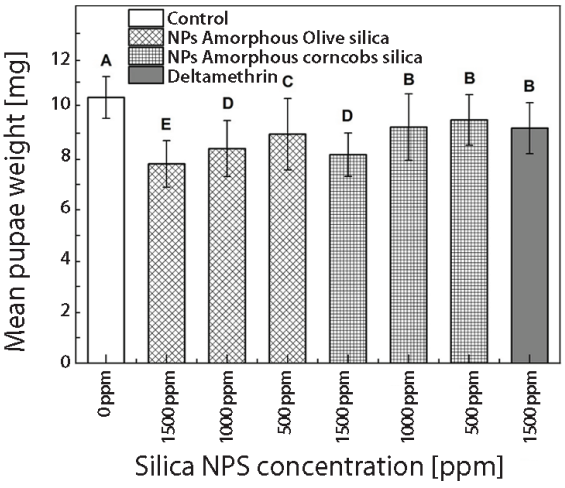


Figure 4. Effect of SiO_2 nanoparticles, derived from corncobs and olive stone, on pupae weight of *Phthorimea operculella.* Different capital letters on top of columns indicate significantly different means at p<0.05 (Tukey HSD test).

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Table 1. Effect of SiO_2 nanoparticles, derived from corncobs and olive stones, on the development time (days) of larvae and pupae of *Phthorimea operculella*.

Treatments	Concentrations	Larval development time (days) (mean ± s.e.)	Pupae development time (days) (mean ± s.e.)
Control	0 ppm	18.2 ± 0.8cd	8.3 ± 0.7c
Olive stone nanoparticles	1500 ppm	21.1 ± 1.0a	9.9 ± 0.9a
	1000 ppm	20.1 ± 1.0b	9.2 ± 1.1b
	500 ppm	19.1 ± 1.1c	8.7 ± 0.7bc
Corncob nanoparticles	1500 ppm	20.8 ± 1.0b	9.2 ± 1.2b
	1000 ppm	18.9 ± 1. 3c	8.9 ± 0.6bc
	500 ppm	18.8 ± 1.0cd	8.8 ± 1.0bc
Deltamethrin	1500 ppm	19.1 ± 3.5c	8.4 ± 0.7c

Means ($\bar{x}$±SE) followed by different capital letters in columns are significantly different at P<0.05 (Tukey HSD test).

most effective the ones from olive stones at 1500 ppm. Further research is needed to determine the influence of morphological and microstructural aspects, such as porosity and crystallinity, on the efficiency of the produced silica nanoparticles against the pest.

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Επίδραση του νανο-πυριτίου που εξάγεται από δύο διαφορετικές φυτικές πηγές στην επιβίωση και την ανάπτυξη των προνυμφών της φθορίμαίας της πατάτας, *Phthorimaea operculella*

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Περίληψη Εξετάστηκε η επίδραση νανο-πυριτίου φυτικής προέλευσης στην επιβίωση και την ανάπτυξη των προνυμφών της φθορίμαίας της πατάτας, *Phthorimaea operculella* (Zeller). Παραλήθφηκε

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σκόνη οξειδίων του πυριτίου από δύο διαφορετικά γεωργικά υποπροϊόντα, τον πυρήνα ελαιοκάρπου και τον σπάδικα αραβοσίτου. Ο χαρακτηρισμός με περίθλαση ακτίνων Χ έδειξε ότι η εκχυλισμένη σκόνη περιέχει μια άμορφη φάση πυριτίου. Οι μετρήσεις προσρόφησης-εκρόφησης αζώτου έδειξαν ότι τόσο το εκχυλισμένο πυρίτιο όσο και το επεξεργασμένο πυρίτιο έχουν μεσοπορώδη δομή, με ειδική επιφάνεια περίπου 300 m^2/g και 270 m^2/g για το πυρίτιο που προέρχεται από τον πυρήνα του ελαιοκάρπου και τον σπάδικα του αραβοσίτου, αντίστοιχα. Τα νανοσωματίδια πυριτίου (SiO_2 NPs) που παρασκευάστηκαν από πυρίτιο που προέρχεται από πυρήνες ελαιοκάρπου είχαν μεγαλύτερη επίδραση στη θνησιμότητα των προνυμφών, στο βάρος των νυμφών και στο χρόνο ανάπτυξης των προνυμφών και των νυμφών της φθοριμαίας, σε σύγκριση με τα νανοσωματίδια πυριτίου από σπάδικες καλαμποκιού. Τα αποτελέσματα δείχνουν ότι το νανο-πυρίτιο που προέρχεται από γεωργικά υποπροϊόντα μπορεί να είναι εξίσου αποτελεσματικό με το συνθετικό εντομοκτόνο (δελταμεθρίνη) που χρησιμοποιείται για την αντιμετώπιση της φθοριμαίας, με μικρότερη περιβαλλοντική επιβάρυνση όσον αφορά στην πρόληψη συσσώρευσης υπολειμμάτων γεωργικών φαρμάκων. Επιπλέον, η αποτελεσματικότητα των επεμβάσεων εξαρτάται από την πηγή προέλευσης των νανοσωματιδίων πυριτίου και την εφαρμοζόμενη συγκέντρωση για να επιτευχθούν τα βέλτιστα αποτελέσματα αντιμετώπισης του εντόμου.

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