Bioassay of *Lantana camara* and *Solanum nigrum* extracts on potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)

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In vitro effect of the aqueous extract concentrations of the methanol crude extract of *Lantana camara* flowers and *Solanum nigrum* fruits were evaluated on the reproduction, feeding response and adult emergence of the potato tuber moth, *Phthorimaea operculella*. Aqueous extracts of the two tested plants hindered the oviposition of gravid females and hatchability. Moreover, the extracts prevented feeding by newly hatched larvae. The extracts caused a decrease in adult emergence from the treated potato tubers as compared to the control. In addition, bioassay results indicate that the extract of *S. nigrum* fruits was more effective than the extract of *L. camara* flowers on the potato tuber moth.

Key words: aqueous extract, oviposition, hatchability, antifeedant, emergence.

INTRODUCTION

Potato, *Solanum tubersum*, is one of the most important economic vegetable crops in Egypt for consumption and export. It is widely spread in Egypt, especially in the northern areas of lower Egypt (Sharaby *et al*. 2009). According to the records of agricultural and economic statistics of the Ministry of Agriculture in Egypt, the total cultivated area in 2018 was 152 523 ha yielded about 4 267 000 t of potato tubers, where 751 840 t of them were for export. Egypt's government is trying to increase potato production, particularly local seed potato, in order to save on imports. The potato crop in Egypt has three growing seasons, called: Nelly; early summer and summer (Ministry of Agriculture 2018). During these seasons the potato plants were attacked by potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), which is considered as one of the most serious pests causing losses in this crop. It attacks the potato tuber in the field and in storage (Ferreire *et al*. 1994). Damage by this pest in suitable conditions during storage is more than in the field (Das 1995).

PTM is oligophagous, feeding only on certain genera of the Solanaceae, but potato is considered the main host plant for this insect in different areas of the world. Females in the field laid higher number of eggs on potato tubers and relatively longer oviposition period than those offered potato leaves (Gomaa *et al*. 1978). Oviposition by PTM increased when given contact with host plant or high levels of host odour (Traynier 1983). Fenemore (1979) showed that eggs are laid freely on suitably textured surfaces in the absence of plant material of any kind.

Classical control of PTM using insecticides caused a high cost and hazardous effects of pesticides on the environment and public health, this makes us seek to use natural means such as the use of plant extracts of some plants against it (Karamanoli 2002). Natural compounds are formed as plant secondary metabolites (allelochemicals) by plants belonging to botanical families like Verbenaceae, Solanaceae, Lamiaceae and Cruciferae. These compounds have functions in chemical defence, acting as repellents, phagodeterrents, growth regulators, insecticides and by attracting natural enemies of herbivores (Bowers *et al*. 1972; Karamanoli *et al*. 2005; Burfield & Reekie 2005; Bakali *et al*. 2008; Elsayed *et al*. 2017). Monophagous and oligophagous species are generally more sensitive to deterrents from non-hosts than polyphagous species.

Alkaloids are nitrogen-containing secondary plant metabolites found in numerous plant species. At least 20 structurally different alkaloids have been recognised in potatoes and tomatoes and about 300 in other Solanaceae species (Schreiber 1979). Glycoalkaloids (α-chaconine and α-solanine) are usually present at low levels in

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Received 23 October 2018. Accepted 24 September 2019

ISSN 1021-3589 [Print]; 2224-8854 [Online]
DOI: https://doi.org/10.4001/003.028.0055

commercial potatoes. However, they can accumulate to high levels in the green and damaged tubers (Mondy & Seetharamam 1990; Friedman & Dao 1990).

The study aims to test two of the plants that have natural chemicals (plant secondary substances) that could be used to reduce the injury of potato tubers in the field or in the stores by *P. operculella*, as well as to reduce the use of pesticides in IPM programmes.

MATERIAL AND METHODS

Insect culture

Moths of *P. operculella* were collected from potato 'Nawala' and were kept in small plastic cages (10 cm in diameter and 15 cm deep) for oviposition. Egg laying cages contained a honey solution (10 %) for adult feeding and fine filter paper as an oviposition site. The cages were covered with black muslin. Collected eggs were placed on non-infested potato tubers in glass jars (1-l), containing a small layer of sand. Newly hatched larvae usually mine inside the tuber and pupation takes place outside on the sand.

Preparation of plant extracts

Flowers of *Lantana camara* (Verbenaceae) and fruits of *Solanum nigrum* (Solanaceae) were collected from the field and then dried. Thirty grams of dry flowers or fruits was ground to powder. The powder was placed in 500 ml of methanol and the mixture was shaken for 48 h using an orbital shaker. The mixture was filtered and the solvent was evaporated to dryness by rotary and electric pump (Chiu 1985). The crude extract was weighed (5.5 g for *L. camara* and 3 g for *S. nigrum*) and then dissolved in 10 ml distilled water to prepare the concentrations for use in the bioassays.

Bioassay of plant extracts

Effect on oviposition

Five pairs of newly emerged adults were inserted in cages (40 × 25 × 20 cm). The cage was made from wire net sides covered by muslin sleeve. Treated and non- treated potato tubers were put in glass Petri dishes (10 cm diameter) and then were placed in each corner of the cage (5 cages as replicates). Three concentrations of flowers or fruits extract (125, 65.5 and 31.3 mg in 0.227 ml, 0.113 ml and 0.056 ml of distilled water, respectively) were assayed and each concentration was sprayed on 100 mg of potato tubers, while the potato tubers in the control were sprayed with distilled water only. Over three successive days, the potato tubers are left with the insects in the test cage, and then the potato tubers were removed and thoroughly examined for counting the eggs deposited by females. The eggs were counted on treated or non-treated potato tubers. Usually the females laid eggs around the buds of potato tubers (Salana *et al*. 1972)

Effect on hatchability and adult emergence

One-hundred eggs from the culture on fine filter paper (2 cm × 1 cm) were treated by topical application at 50, 25 and 12.5 mg of crude extract in 0.090 ml, 0.045 ml and 0.0225 ml of distilled water, respectively. Each concentration was replicated five times. Treated or non-treated eggs were placed in different glass jars (1-l) each containing 50 g of potato tubers. Hatchability was calculated (Elsayed & Al-Otaibi 2006).

$$\text{Hatchability} = \frac{\text{No. of hatched eggs}}{\text{No. of treated or non-treated eggs}} \times 100$$

Hatched larvae were maintained on potato tubers until the life cycle was completed and the adults had emerged.

Effect on feeding response behaviour

Fifty newly hatched larvae were put in the middle of a glass jar (2-l) containing treated potato tuber (100 mg) in one corner at different concentrations of crude extract (125, 62.5 and 31.3 mg in 0.416 ml, 0.208 ml and 0.104 ml of distilled water, respectively), and another corner containing non-treated potato tubers. Five cages were used for each concentration. Three days after treatment the potato tubers treated or non-treated at different concentrations were transferred into separate glass jars (1-l) for counting the emerged adults.

$$\text{Feeding response \%} = \frac{\text{No. of emerged adults}}{\text{No. of newly hatched larvae}} \times 100$$

All experiments were carried out under laboratory conditions of 25 °C and 71.5 % R.H. (Gomaa *et al*. 1978). All experiments were statistically analysed by *t*-test method (Waller & Duncan 1969).

RESULTS

Oviposition behaviour

Under laboratory conditions Table 1 indicates a significant ovipositional-deterring effect of

Table 1. Effect of *Lantana camara* and *Solanum nigrum* extracts on ovipostion behaviour of *Phthorimaea operculella*.

Plant extract concentrations*:	Mean number of deposited eggs								
	125 mg	Control	*t*-test values $P \leq 0.05$	62.5 mg	Control	*t*-test values $P \leq 0.05$	31.3 mg	Control	*t*-test values $P \leq 0.05$
L. camara	30.0	99.8	79.96	49.2	89.6	17.98	56.4	68.8	10.27
S. nigrum	25.4	84.2	42.00	39.0	80.4	25.87	49.0	72.8	7.00

*mg/crude extract (g).

L. camara and *S. nigrum* extract. Mean number of eggs deposited by females was significantly higher on non-treated potato tubers than those on treated and the effect was directly related to the concentrations of the extract.

Hatchability and adult emergence

The percentage of hatching and adult emergence decreased with the increasing concentration of the extract of *L. camara* and *S. nigrum* treatment (Table 2). The hatchability of treated eggs by *L. camara* extract was less than that in the control treatment and the significance was higher and inversely related to concentrations of 50 and 25 mg, but no significant difference was recorded when the 12.5 mg concentration was used. The percentage of emerged adults from treated eggs at high concentrations (50 and 25 mg) of the extract was significantly lower. However, the total adult emergence with 12.5 % of the extract was almost equal with the control and no significant difference was recorded.

The fruit extract of *S. nigrum* showed a strong effect on both egg hatching ratio and total adult emergence ratio at all tested concentrations, with highly significant difference between all treatments and control (Table 3).

Feeding response

Feeding response of newly hatched larvae on treated potato tuber of different concentrations (125, 62.5 and 31.3 mg) of *S. nigrum* extract was significantly lower than that in non-treated potato tubers. This result was reflected in the rate of development of larvae to adults (Table 4). The flower extract of *L. camara* at 125 and 62.5 mg have

Table 2. Effect of *Lantana camara* extract on hatchability and number of adult emergence *Phthorimaea operculella*.

Concentrations	Hatchability %			Adult emergence %		
	L. camara	Control	*t*-test values $P \leq 0.05$	*L. camara*	Control	*t*-test values $P \leq 0.05$
50.0 mg	54.8	85.6	71.4500	60.0	83.6	17.3500
25.0 mg	78.6	92.4	6.0400	77.6	93.7	4.8588
12.5 mg	84.0	83.6	0.0528	85.9	87.6	0.1482

mg/crude extract (g).

Table 3. Effect of *Solanum nigrum* extract on hatchability and number of adult emergence *Phthorimaea operculella*.

Concentrations	Hatchability %			Adult emergence %		
	S. nigrum	Control	*t*-test values $P \leq 0.05$	*S. nigrum*	Control	*t*-test values $P \leq 0.05$
50.0 mg	12.2	87.8	65.80	31.1	90.9	95.00
25.0 mg	29.4	86.6	21.12	32.7	91.9	50.00
12.5 mg	32.6	89.6	38.00	46.6	88.8	21.56

mg/crude extract (g).

Table 4. Effect of *Lantana camara* and *Solanum nigrum* extracts on feeding response of newly hatched larvae of *Phthorimaea operculella*.

Plant extract	Plant extract concentrations								
	125.0 mg	Control	*t*-test $P \leq 0.05$	62.5 mg	Control	*t*-test $P \leq 0.05$	31.3 mg	Control	*t*-test $P \leq 0.05$
	Feeding response %								
L. camara	16.0	72.0	22.13	23.6	57.2	7.67	46.8	48.8	0.15
S. nigrum	7.2	73.2	55.00	19.6	63.2	8.40	65.6	65.6	43.20

Plant extract	Plant extract concentrations								
	125.0 mg	Control	*t*-test $P \leq 0.05$	62.5 mg	Control	*t*-test $P \leq 0.05$	31.3 mg	Control	*t*-test $P \leq 0.05$
	Mean of adults emergence								
L. camara	8.0	36.0	22.13	11.8	28.6	7.67	23.4	24.0	0.15
S. nigrum	3.6	36.6	55.00	9.8	31.6	8.40	11.2	32.8	43.20

an antifeedant effect on larvae and adult emergence of *P. operculella*, but with 31.3 mg extract the emergence ratio of adults did not record any difference between treated or non-treated potato tubers. The percentage of feeding response at 31.3 mg was 46.8 % on treated potato tubers, while it was 48.8 % on non-treated potato tubers (Table 4).

DISCUSSION

Results of oviposition behaviour of *P. operculella* females on treated potato tubers with *L. camara* flower and *S. nigrum* fruit extracts, showed a high impact and the effect was noted to be dependent on the concentration. Such an effect may be a chemical excitatory cue omitted by the plant extracts and acted as an oviposition repellent. Similarly, *L. camara* and *Minthostachys* sp. had potato tuber moth repellent properties which reduce sprout damage, besides the physical barrier effect resulting from covering stored tubers with rice or barley straw (Raman *et al*. 1987).

Insecticidal plants can inhibit insect feeding and oviposition (Mordue & Blackwell 1993). Dried powders of *Allium cepa*, *Curcuma longa*, *Colocasia antiqurum*, *Ocimum basilicum*, *Dodonaea viscosa* and *Thuja orientalis* played a highly significant role in reducing egg deposition by *P. operculella* (Sharaby *et al*. 2009). Ovipostion by the flea beetle *Longitarsus bethae* on the root of *Lantana camara* was significantly reduced at high densities of the bug, *Teleonemia scrupulosa* nymphs on the leaves of *L. camara* (David 2006). Essential oils (EOs) of basil, European pennyroyal, lavender, mint, oregano and savoury elicited the highest reduction in percentage of egg laying by *P. operculella* (Rafiee-Dastjerdi *et al*. 2014). Oviposition deterrent activity of *Spodoptera litura* was recorded with three plant extracts, *Aegle marmelos* (ethyl acetate extract), *Cinnamomum zeylanicum* (hexane extract) and *Ocimum americanum* (ethyl acetate extract) (Arivoli & Samuel 2013).

However, adult females seem to be less dependent on plant odour for oviposition, and water-soluble leaf chemicals appear to be major stimulants in some instances (Elsayed 2011). The plant cuticle is a highly complex lipophilic structure which is composed of the cuticle layer and it has an effect on the oviposition of the lily leaf beetle, *Lilioceris lilii* (Muller & Riederer 2005). Aquatic plant substrate is an important parameter for oviposition choice by damselfly females (Zygoptera and Odonata) (Nataly & Stanislav 2007). Phenolic compounds deposited on the surface of cabbage leaves, as well as the release of volatiles, repelled insect pests (Lynch & Zobel 2002). These data explain the fact that the plant allelochemicals influence the chemosensory behaviour of the gravid females of *P*. *operculella*.

Hatchability percentage of eggs of *P. operculella* treated with aqueous extract of *L. camara* and *S. nigrum* is affected by concentration rate, as the concentration increases hatchability decreases. The bioactivity of plant extracts sprayed on *P. operculella* eggs may be attributed to allelochemicals in crude extracts having the ability to penetrate eggs and prevent the development of the embryo. Eggs of *P. operculella* did not hatch when they were dipped in undiluted vegetable oils (Raman *et al*. 1987). Aqueous extracts of *Alibertia intermedia* and *Alibertia sessilis* caused the lowest fecundity of *Plutella xylostella* (Lucas *et al*. 2017). Fertility of *Euprepocnemis plorans* was affected on *Lupinus termis* and *Vicia faba* diets (Elsayed 1998). In the light of the results obtained from this work, *L. camara* and *S. nigrum* aqueous extracts may have an insecticidal effect against the larvae of *P. operculella*, which resulted in decreasing the emergence of adults. High mortality of fourth and fifth larval instars of *P. operculella* was noted after the larval stages were treated with plant extracts of black pepper (*Piper nigrum*) and Hungarian chamomile (*Matricaria chamomile*) (Najat 2010). Ethanolic extracts of *Pithuranthos tortosus* and *Iphiona scabra* caused the maximum inhibition of egg hatchability of *P. operculella* (Sharaby 2009).

Feeding response of newly hatched first instar larvae between treated and untreated potato tubers showed that the crude extract of *L. camara* and *S. nigrum* has an anti-feeding effect, and in host selection the larvae preferred untreated tubers. This action of the crude extracts may be attributed to allelochemicals and their degradations as solanine alkaloids in *S. nigrum*. Secondary plant metabolites (allelochemicals) play a major role in plant-insect interactions (Elsayed 2011). Potato tubers treated with methanolic extract of lavender elicited the lowest percentage (19.3 %) of first instar larval penetration of *P. operculella* (Rafiee-Dastjerdi *et al*. 2013). Phenolic compounds deposited on the surface of cabbage leaves as well as the release of volatiles repelled insect pests (Lynch & Zobel 2002). Monophagous and oligophagous species are generally more sensitive to deterrents from non-hosts than polyphagous species. This has been documented for locusts (Bernays & Chapman 1994). Larvae of *Ceratitis capitata* rejected the diet containing quinine sulphate (Crisci & Zucoloto 2001). Leaves of *Cedrela odorata* that contained limonoids had fewer meals taken by the weevil *Exophthalmus jekelianus* than did leaves without limonoids (Wright *et al*. 2003). Toosendanin at 56.6 ppm showed 50 % feeding inhibition of third instar larvae of *Helicoverpa armigera* (Opender *et al*. 2001). Syringin and chlorojanerin from *Rhaponticum pulchrum* were shown to be good anti-feedants against three species of stored product insect pests *Sitophilus granarius*, *Trogoderma granarium* and *Tribolium confusum* (Joanna *et al*. 2006). Ethyl acetate extract of *Hygrophila auriculata* at 5.0 % concentration had higher anti-feedant (68.48 %) of *Spodoptera litura* (Baskar, 2011). *Aesculus hippocastanum* extract had a strong antifeeding activity of *Lymantria dispar* (Sonja 2012).

The results also indicate that the crude extract of *S. nigrum* were more effective on *P. operculella* than the *L. camara* extract. It is possible the allelochemicals of *S. nigrum* contain solanidine which has more diverse effects on insect behaviour and physiology than the allelochemicals (monoterpenes, germacrene d, 3-elemene, β-caryophyllene, β-elemene,α-copane, and α-cadinene) of *L. camara* (Murugesan *et al*. 2016).

Wild weed *S. nigrum* has the same allelochemicals as in potato tubers (*Solanum tubersum*), but the former contains higher concentrations of solanidine than in potato tubers and/or it produces more toxic glycosylated derivatives such as, α-chaconine and α-solanine. Such products which have no adverse phytotoxic effects on stored tubers may be used successfully in preventing the attacks by *P. operculella* on tubers in the store.

CONCLUSIONS

This study was concerned with the effect of plant extracts of two plants containing secondary plant substances (allelochemicals) in the control of potato tuber moth in storage and in the field. The results of laboratory tests showed that both *S. nigrum* and *L. camara* extract plants have an effective effect on insect behaviour, which reduces their hazardous effect on potato crop productivity.

The study recommends the importance of using a plant extract as a bio-insecticide for *P. operculella* in IPM programmes in the future to reduce the costs of pesticide use and reduce environmental pollution.

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