Entomotoxicant potential of arak shoot extract for the control of *Ephestia cautella*

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ABSTRACT

The date, palm, *Phoenix dactylifera* is the most significant agricultural product in the Middle East region, in which Saudi Arabia is ranked as the top producing country. One of the most critical problems facing date production is insect infestation. *Ephestia cautella* is one of the most harmful insects to date, causing significant damage. Different techniques have been used against *E. cautella* through the use of synthetic chemical pesticides, but they have a negative impact on the environment. Recently, research has been directed towards seeking alternatives to these methods. In the current study, a biological system using *Salvadora persica* shoot extract for the control of *E. cautella*has been evaluated. To this end, mortality rates LD_{50} and LD_{90} were determined when the different concentrations of the plant extract were applied at different developmental stages (larvae, pupa, and adult male/female) over a period of three days. Furthermore, some chemical constituents of the plant extract were determined by preliminary chemical tests and by using Gas chromatography–mass spectrometry (GC-MS). Results from current investigation have displayed that the mortality rate was dependent on both the developmental stage and concentrations of the plant extract. Total phenolics and the saponin content of the plant extract were detected. GC-MS analysis identified the potential chemical agents in the plant extract that may possess the insecticidal ability. In light of the current findings, it is clear that infestation of date palms by *E. cautella* can be controlled by using *S. persica* shoot extract. To optimize the implementation of this technique, we recommend that future research focus on testing other organic solvents and other plant parts so that the most biologically active biomolecules responsible for the control of *E. cautella* under date storage conditions can be accurately determined.

Keywords: plant extract; *Salvadora persica*; *Ephestia cautella*; date palm; GC-MS

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INTRODUCTION

The date, palm (*Phoenix dactylifera*) is known as one of the most important types of horticultural trees, producing dates which are well known for their high nutritional value relative to other types of fruits. This tree also has religious significance, as it has been cited in the holy Quran for Muslims. The good climactic conditions of the Middle East region are favorable for the growth of date palm. Saudi Arabia is ranked one of the top date-producing areas (Alabdulhadi *et al*., 2004) and in 2008 the kingdom's production of date amounted to 980,000 tons (AOAD 2009). Generally, insect pests constitute the most critical obstacle to human development in connection with food security, especially in countries whose economies rely largely on agriculture and natural resources. The most serious problem facing date production is the infestation by insects under storage conditions in warehouses. *Ephestia cautella* is considered one of the most destructive insects with respect to stored food materials, of which the date is no exception (Singh and Moore, 1985), with consequent substantial losses. Such damage may have a significant effect on the food security of the world population, particularly in countries that cannot afford to provide sufficient facilities to control these insects. Moreover, the danger of infestation by insect pests is principally due to the fact that insects reproduce and multiply easily in very short time periods, which gives them the strong potential to cause severe infestation of a wide range of agricultural products both in fields as well as in storage conditions (Akinneye and Ogungbite, 2016). This situation has led to increasing concern in the agricultural sector world-wide, resulting in considerable efforts to find the most appropriate and effective techniques for fighting destructive insects endangering agricultural products all over the globe.

The majority of these techniques basically depend on chemically synthesized compounds, which, however, highly efficient, have some practical limitations (Mulla and Su, 1999). Firstly, the utilization of insecticides from synthetic chemical is expensive and toxic to the environment. In cases of intensive use and long-term exposure to such synthetic chemical insecticides, some health issues may emerge which critically affect human health, such as irritation and allergic dermatitis (Reynolds, 1994). Furthermore, previous reports have clearly indicated the inefficiency of using synthetic insecticides for insect pest control as the insects have developed resistance to these synthetic insecticides (Ranson *et al*., 2001). Because of these limitations, recent research has shifted towards finding other alternatives to replace those chemical techniques with others that are less costly while being more suitable, acceptable to humans and environmentally friendly. Generally, use of insecticides produced from plant origin has verified as a dependably effective approach for insects control, while such method is environmentally harmless, readily accessible, cheap, and the extract employed is degradable and target-specific (Hodges *et al.*, 1984; Pitasawat *et al.*, 2005). Numerous experiments have been achieved on the extracts of various plant parts such as the fruit, leaves and roots of some plant species to explore their biological activity as possible alternatives to the synthetic chemical agents for controlling insects (Al-Sharook *et al.*, 1991; Murugan and Jeyabalan, 1999; Muthukrishnan and Puspalatha, 2001; Senthil and Saeboon, 2006; Olayemi *et al*., 2014; Misni *et al*., 2016; Norris *et al*., 2015; Sathantriphop *et al*., 2015). Other similar studies have provided sufficient evidence that insecticides of plant origin extracted from some candidate plant species can successfully be used as good alternatives to the synthetic chemical insecticides Mittal and Subbarao, 2003; Rajkumar and Jebanesan, 2004). The efficacy of insecticides derived from plants has been associated with the presence of bioactive chemicals, which inhibit the growth (Sharma and Srivastava, 2006) and metamorphosis of insects (Mwangi and Rembold, 1986; Kumar *et al.*, 1991) and these bioactive substances may be present in single or combined forms. The bioactive compounds are natural insecticides obtained from plant origin that help the plant to withstand different environmental factors (Ghosh *et al*., 2012). In early studies, Hodges *et al.* (1984) reported that *Ephestia cautella* can be controlled using synthetic sex pheromones both under laboratory and storage conditions.

Salvadora persica is one of the most important trees of the *Salvadoraceae* family. It is a large shrub, about 3m high, which sometimes grows as dense thicket on sand hills. The species is widely distributed, from Africa through the Arabian Peninsula to China. *Salvadora persica* is cited in the holy Quran and Sonnah and for a long time its roots and shoot sticks have been used as oral hygiene tools (toothbrush, *miswak*) in many parts of the globe. Previous findings have indicated the potency of *S. persica* aqueous extract as an anti-microbial (Almas, 2001) anti-mycotic (Al-Bagieh *et al*., 1994) agent as well as for pest control (Sher, *et al.*, 2010). Alkaloids, terpenoids, flavonoids, steroids, saponins and volatile oils are the biologically active ingredients in S. persica (Garboui *et al*., 2009; Abdillahi *et al*., 2010; Ahmad and Rajagopal, 2013). Considerable tick-repellent efficacy by *S. persica*has been well documented (Garboui *et al*., 2009). Different chemical compounds have beenisolated from *S. persica such as* salvadoricine (a new indole alkaloid from the leaves (Malik *et al*., 1987), lignin glycosides, quercetin and the flavonoid rutin from the stems(Ohtani *et al*., 1992; Abdel-Waheb *et al*., 1990), and salvadourea and benzyl isothiocynate from the root (Ray *et al*., 1975; Al-Bagieh, 1990).Despite the previous data published on controlling insect pests in general, few studies have been performed with respect to the toxicological impacts of *S. persica* and its effects on date-infecting insects. Therefore, the current investigation has been carried out in an attempt to evaluate the potential of using the plant extract from *Salvadora persica* as a possible alternative treatment to control *Ephestia cautella*, the most harmful and destructive insect to the date palm in the Kingdom of Saudi Arabia. To attain these target mortality rates, LD_{50} and LD_{90} were determined and evaluated.

MATERIALS AND METHODS

Insects

E. cautella adults, pupa and larvae were used in this investigation. Rearing of the insects was done on wheat germs under laboratory conditions in the department of Biology, Faculty of science, King Saud University. Cultures were held at 25°C with 65 ± 5% relative humidity under 16 hrs of light and 8 hours of darkness.

Plant Materials

Fresh leaves from *Salvadora persica* were collected from south Saudi Arabia, washed with distilled water and dried in shade then ground to fine powder. About 10 g of the dry powder were submitted for extraction and added to 100 mL ethanol (80%) and incubated for24-hr period. Thereafter, mixture was filtered and heated at 240°C for ethanol evaporation then kept at 4 °C for further usage and this procedure was repeated three times for replication.

Biological Study

The insecticidal efficiency of the *Salvadora persica* leaf extract was experienced at six different concentrations against *E. cautella* in different developmental stages. In total, 20 larvae and 20 pupa, as well as 20 adult males and 20 adult females) were transferred to the treatment containers that had the wheat germs soaked with 100, 75, 50, 25, 12.5 and 6.25% plant extract for three days. Closed containers with a sufficient air supply were kept at 25°C with 65 ± 5% relative humidity. The survival numbers of larvae and pupae as well as the adults (both male and female) were considered. The number of insects in the different tested stages were recorded to determine the mortality rate in each applied concentration. Distilled water was used as negative control. The number of dead insects in the different stages were recorded every day for three days, monitoring the effects of the different extract concentrations. Thereafter, insects were transferred to fresh untreated wheat germs and observed. The total number of surviving insects was noted. The study was replicated three times. The percent mortality data after corrections were subjected to profit analysis for calculation purposes. The percentage of the mortality rate was corrected according to Abbott's formula below (Abbott, 1925):

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae} \times 100}{\text{Number of larvae introduced}}$$

Plant Chemical Components Analysis

The presence of alkaloids (Joshi *et al*., 2013), saponins (Harborne, 1998) and total content of phenolic compounds (Joshi *et al.*, 2013) were estimated.

GC-MS Analysis of the Plant Material

Ethanol has been applied to dissolve the plant extract, and the combination has been detected using the GC-MS SHIMADZU QP2010 (Shimadzu, Kyoto, Japan) instrument with Elute DB-5M column. Oven temperature (70 °C) has been used for 2.0 minutes and afterward increased gradually up to 300°C. Helium (99.99%) was used as carrier gas as well as eluent. For a duration of 35 minutes, 70 eV and 40-1000 m/z were applied. Phytochemical constituents have been recognized according to their mass spectra.

Statistical Analysis

Data in the current investigation were processed statistically and the analysis has been completed by the JMP software package (version18.0) with variance analysis (one-way ANOVA). The statistical variations among the treatments have been indicated by the Duncan multiple range test ($p \leq 0.05$). Data has been calculated and stated as mean ± standard deviation for three

Table 1. The effect of the *Salvadora persica* leaves extract at different concentrations (6.25, 12.5, 25, 50, 75, 100%) on *Ephestia cautella* exposed for 48 hours at different developmental stages.

Stage	**Concentration (µg/ml)**						**LD_{50} (µg/ml)**	**LD_{90} (µg/ml)**
	Mortality of developmental stage %							
	6.25	12.5	25	50	75	100		
Larva	1±0.3	10±0.2	20 ±0.1	49.3±0.9	46.7±0.2	100±0	50.3	90.2
Pupa	1±0.4	11±0.5	22 ±0.4	50.3±0.1	73.3±0.3	100±0	51.3	95.5
Adult female	5±0.1	19±0.8	50 ±0.3	43.3±0.6	100±0	-	55.9	85.9
Adult male	10±0.2	16±0.2	60.3 ±0.7	73.3±0.1	100±0	-	33.8	75.8

replicates per treatment. Mortality rate data were used to compute the mean lethal concentrations (LC_{50}) and (LC_{90}) of the plant extract (Finney, 1971).

RESULTS

The current study has been assumed to estimate the influence of the bioassay of the *S. persica* leaves' ethanolic extract at different concentrations on *E. cautella*. Results from this study represent the LD_{50} and LD_{90} levels that were expressed as mg/mlin all the tested stages of *E. cautella* when subjected to the plant extract (Table 1). Different susceptibility levels among different developmental stages were observed. Adult male and female categories were the most sensitive stages with LD_{50}level at 48 hours at concentrations of 33.8 and 45.9 µg/ml, whereas the LD_{90}concentrations were 75.8 and 85.9 µg/ml, respectively. Furthermore, for pupa, which was the most tolerant stage, the LD_{50}concentration was 55.3 and the LD_{90}concentration was 95.5 µg/ml. The order of tolerance of the different stages at both LD_{50} and LD_{90} levels was: pupa > larva > adult female > adult male. No mortality rate was observed in the control. Our results revealed that the mortality rate for each stage was incremental to the increase in the exposure time since a 100% mortality rate was observed after 3 days in the concentrations of 75 and 100%, but an increment of the concentration also resulted in the increase of the mortality rate.

The mortality rate of *E. cautella* that were exposed to ethanolic plant extract in their different developmental stages was significantly higher than that of the control (Table 1) indicating the ability of *S. persica* to perform insect control.

The preliminary phytochemical test indicated that the plant extract contains phenolic (25 µg/mL) and saponins, but that alkaloids are absent. Some chemical components that might be responsible for more insecticidal ability of the plant extract were isolated and identified from the ethanolic plant extract by the application of the GC-mass system. It has been found that *S. persica* leaf extract possesses a wide range of chemical compounds that are presented in (Table 2). A total of 13 components were identified by their retention time (RT). Cyanazine, bentazone, boric acid,2-butanol, fenipentol, benzyl alcohol, phenol,3 methyl, and testosterone are some examples of the isolated chemicals from the ethanolic extract of *S. persica*.

DISCUSSION

The notable alternative to the synthetic chemical in insect control is the use of biotic material such as phytochemicals that have been found in plant extracts (Alkhalifa, 2013). Plant are accessible and their extracts easy to apply. Also, they are safe with no harmful effects on the environment.

The major objective of this study was to determine chemical insecticides of plant origin since the tropical areas of the world have medicinal plants that might have insecticidal ability (Ofuya, 2001; Akinkurolere *et al*., 2006). A recent study confirmed that secondary plant metabolites may serve as defense mechanisms against insects(Rizwana *et al.,* 2016).Such compounds with insecticidal ability are alkaloids, steroids, terpenoids, essential oils, and phenolics(Short and Colborn, 1999).

Derivatives from botanicals can be isolated by different extraction methods and therefore the solvent used for the extraction of the effective components has an effect on their efficiency (Ashamo and Ogungbite, 2014). Oils from different plants such as *Capsicum frutescens*,

Table 2. The chemical compounds detected by GC-MS analysis of ethanolic extract of *Salvadora persica*

Compound name	Retention Time	Molecular Formula	Molecular Weight
Benzeneacetic acid, alfa.-hydroxy-, ethyl ester	3.18	$C_{10}H_{12}O_3$	180
Benzenemethanol,.alfa.- (ethoxymethyl)	3.18	$C_{10}H_{12}O_2$	166
Benzenemethanol,.alfa.- ethyl	3.18	$C_9H_{12}O$	136
Propylene Glycol	4.07	$C_3H_8O_2$	76
2-Butanol	4.07	$C_4H_{10}O$	74
Boric acid	4.07	BH_3O_3	62
Ergoline-8-carboxamide,9,10-didehydro-6-methyl-, (8.beta)-	44.35	$C_{16}H_{17}N_3O$	267
Bentazone	48.01	$C_{10}H_{12}N_2O_3S$	240
Cyanazine	48.01	$C_9H1_3CIN_6$	240
Fenipentol	3.18	$C_{11}H_{16}O$	164
Benzyl alcohol	3.18	C_7H_8O	108
Phenol,3 methyl	3.18	C_7H_8O	108
Testosterone	59.19	$C_{19}H_{28}O_2$	288

Anacardium occidentale, *Monodora tenuifolia*, *Xylopia aethiopica*, and *Ricinus communis* were found to be effective and recommended against *E. cautella* when different stages of the insect were tested (Akinneye and Ogungbite, 2013).

Regarding the effect of the different concentration of the plant extract, a similar study by Akinneye and Ogungbite (2016) on the activity of some medicinal plant against *Ephestia cautella* has documented that the mortality rate varied according to the dosage used. Regarding the relative tolerance of insect developmental stages, a previous finding has indicated that pupa is the most tolerant stage when the toxicity of fumigant propylene oxide was examined against *Tribolium castaneum* (Navarro *et al*., 2005). In a trial to isolate and identify the exact chemical components in *S. persica* that might be responsible for the insecticidal effect, phenol and saponin were detected and therefore, the demonstrated insecticidal activity might be attributed to their presence since phenolic compounds may inhibit insect development (Sharma *et al.,* 2006) and metamorphosis (Mwangi and Rembold, 1986; Sukumar *et al.,* 1991). Furthermore, saponin was isolated from *Balanites aegyptiaca* (Chapagain *et al*., 2008), which had insecticidal ability. A test to isolate the exact chemical components that might be responsible for the insecticidal effect using the application of GC-mass technique was the key focus of this study.

Isolated cyanazine is a triazine herbicide and pesticide that has been widely used in some agricultural parts of the United States(Kolpin *et al*., 1998) and has been identified in surface and ground waters (Dorfler *et al*., 1997; Dorfler *et al*., 2011). Furthermore, bentazone is an effective herbicide that is toxic for marine organisms (EPA, 2006). Moreover, propylene glycol is usually used in air sanitization and hard surface disinfection (Habes *et al*., 2006). On the other hand, when boric acid was assessed against the German cockroach, *Blattella germanica* L., high insecticide ability was detected (Pravin *et al*., 2011).

Fenipentol, benzyl alcohol, testosterone, benzene acetic acid, and benzene methanol, alpha-(ethoxymethyl) were also isolated from ethyl acetate and methanol extract of the *Eruca sativa* seed that showed high antimicrobial action (Rizwana *et al*., 2016).Benzyl alcohol is one of the most active oil components of *Cinnamomum porphyrium* and *Aloysia citriodora* which were effective head lice repellents (Toloza *et al*., 2010). In the present study it is clear that the leaf extracts of *S. persica* L (Araak) have some effectiveness against *Ephestia cautella*. Such activity might be related to the chemical isolated constituents such as cyanazine, bentazone, fenipentol, benzyl alcohol,testosterone, benzene acetic acid, benzene methanol, and alpha-(ethoxymethyl) and benzyl alcohol that showed effectiveness against insects and microbes in different studies. Furthermore, some potency of *S. persica* was documented against larvae of *Culex quinquefasciatus* (Hammed, 2015).

Commonly, it is well known that the secondary metabolites of plants are harmful constituents that prevent the plant from enemies. The negative impact on the insect that the phytochemicals might affect the insect physiological behavior, for instance the abnormalities in the nervous system since they have the ability to interfere with the mitochondria and render their proper functioning (Rattan, 2010).

The present findings providing indication on the capability of *S. persica* shoot extract as an insecticidal for *Ephestia cautella*. Additional investigations could help in optimizing usage of *S. persica* shoot extract for improving extract preparation that allow to be applied for insects control in storage conditions.

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Competing Interest

The authors have declared that no competing interest exists.

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