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Original article

Impact of the invasive plant species "*Nicotiana glauca*" toxins on the larvae of the invasive insect species "*Rhynchophorus ferrugineus*": A damaging pest of date palm trees in Saudi Arabia

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ABSTRACT

The wild tree tobacco (Nicotiana glauca) is an alien species that invaded vast areas of the Southwestern region of Saudi Arabia. While, the Red Palm Weevil (RPW) (Rhynchophorus ferrugineus) is considered to be the most damaging invasive insect species of palm trees all over the kingdom of Saudi Arabia, causing major economic losses to farmers and the economy of the country. Using conventional insecticides to control harmful insects such as RPW has undesirable effects on the environment and human health. Alternatively, using biocontrol agents such as poisonous extracts from N. glauca might be a better approach in pest management and can be considered as an eco-friendly, cost-effective, and safe alternative. Therefore, the current study aimed to evaluate the larvicidal effect of N. glauca aqueous extracts against the red palm weevil larvae. The plant specimens were collected from Al-Baha region in the Southwest of Saudi Arabia. Each single test consisted of 20 larvae, and N. glauca preparations were; 1, 1.5, 2, 2.5, and 3 ml, besides the control test. Results obtained for the effect of botanical extracts; leaf, flower, stem and root against R. ferrugineus larvae for an exposure period of 24 hr. at the concentrations of 2.8, 4.2, 6.0, 7.0 and 8.0 ppm. The concentrations for *N. glauca* extracts reflected an LC₅₀ of 2.7 ppm for leave, 2.6 ppm for flower, 2.8 ppm for stem and 7.00 ppm for root. While, the same concentrations extracts reflected an LC₉₅of 11 ppm for leaf, 9.6 ppm for flower, 8.9 ppm for stem and 13.00 ppm for root. These results showed that N. glauca extracts have a remarkable potentiality as insecticidal substances that can be used as an ecofriendly integrated approach for the management of R. ferrugineus. © 2020 The Author. Published by Elsevier B.V. on behalf of King Saud University. This is an open access

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1. Introduction

The wild tree tobacco (*Nicotiana glauca*) occupies vast areas in the Southwestern region of Saudi Arabia. It is a fast growing exotic plant species that has become an invasive weed in that region over the last few decades (Alshahrani, 2008). N. *glauca* is well documented as a toxic plant, however, it has been used medicinally where leaves are normally warmed and then applied to the head in order to relieve headache, and can be applied on the throat to relieve sore throat and on shoes in order to cure painful feet. *Nicotiana glauca* belongs to the family Solanaceae that has been known

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for decades with the presence of pyridine alkaloid of nicotine and anabasine which are very toxic to herbivores and humans (Baldwin & Callahan 1993), (Mizrachi et al, 2000), (Panter et al, 2000) and (Soberon et al 2007). Anabasine is well documented to be very toxic alkaloid to animals including insects (Keeler et al., 1981). But, recently, this alkaloid has been recognized as a source of potential insecticide substances. It can be used in minor concentrations in insect traps as an effective natural insecticide (Zammit et al, 2014).

Red Palm Weevil *Rhynchophorus ferrugineus* (RPW) is considered as the most damaging invasive insect species of palm trees in Saudi Arabia, which causes major economic losses to farmers and the economy of the country. The first record for this insect in the Arabian Gulf countries was in 1986 in the United Arab Emirates; then it appeared in Saudi Arabia in 1987 (El-Juhany, 2010). *R. ferrugineus* has the widest geographical distribution worldwide among the genus of Rhynchophorus weevils and is it is well known as a destructive pest of entire farms of cultivated date palms (Al-Dosary, et al, 2015).







Biological control agents against R. ferrugineus have been explored for decades where many studies have evaluated the potentiality of existing predators and parasites that can eliminate the aforementioned insect. For example, Mahmud et al, (2015) reported an incident when Platymerus laevicollis was introduced to Sri Lanka from Western Samoa as a potential predator of Oryctes rhinoceros and instead it was discovered to prefer R. ferrugineus. In addition, Chelisoches morio nymphs was reported to consume 5.3 R. ferrugineus weevil eggs and 4.2 weevil larvae per day, whereas, adult insects of C. morio consume 8.5 weevil eggs and 6.7 weevil larvae per day (Abraham and Kurian, 1973). Also, oil derivative from garlic and its synthetic form diallyl disulphide were found to be toxic to RPW weevil. Hallett et al. (1999) described how pheromones can be used as a biocontrol against R. ferrugineus and detailed protocols were described for pheromone-based mass trapping of the weevil. Pheromone lures for the RWP weevil in palm date farms in Saudi Arabia have been evaluated nearly two decades ago and outcomes showed that high release lures (Ferrolure and Ferrolure+) obtained from ChemTica Natural, Costa Rica, have the potential to attract twice as many weevils as low release lures (Faleiro et al. 1999). Therefore, it is well documented that botanical materials are important source for insecticides (Isman, 2006). Accordingly, the current study aimed to investigate the larvicidal effect of Nicotiana glauca aqueous extracts against the red palm weevil larvae.

2. Materials and methods

2.1. Plant material

Fresh *N. glauca* specimens were collected from Al-Baha region in the south-west of Saudi Arabia. Specimens of leaves, stems, flowers, and roots were chopped and spread on a tarp, and allowed to air dry, and then further ground to pass through a 2.4 mesh screen using a Gehl Mix-All model 55 (Gehl Company, West Bend, WI, USA). The dry ground plant material was stored carefully in polyethylene bags in the dark at ambient temperature prior to use.

2.2. Red palm weevil

Red palm weevil larvae were collected from high infested palm tree farms that not received any chemical treatment such as insecticides, and then larvae were kept in a glass jar that contained small pieces of sugar cane.

2.3. Bioassay

Each single test consisted of 20 larvae of *Rhynchophorus ferrug-ineus* which was taken into 500 ml beaker that have a certain volume (150, 100, 200, 250 and 300 ml) of dechlorinated tap water PH (7.4). *N. glauca* preparations were added with the following concentration; (1, 1.5, 2, 2.5, and 3 ml), besides dechlorinated tap water as a contrl. Each test was based on three replications. The perished larvae was counted and recorded after 24 h of exposure.

2.4. Data analysis

After 24 hr. exposure time, the dead larvae were counted. The data were plotted in a table organized to show the obtained percentage mortalities (tested and corrected), the concentrations of the *N. glauca* preparation used (in ppm and their corresponding log's), and the probit values (tabulated and calculated) to determine the lethal concentrations (LC_{50} and LC_{95}).

3. Results

Active insecticidal phytochemicals which extracted from plants are becoming increasingly important in research as a result of increased concern for the health and environment resulting of long use of conventional synthetic insecticides. Therefore, using biocontrol agents such as poisonous extracts from *N. glauca* might be a better approach in pest management with less harm to both environment and human health. Accordingly, the current study aimed to investigate the larvicidal effect of *N. glauca* aqueous extracts against the red palm weevil larvae and the results are shown in the (Table 1, Table 2 and Table 3) and (Fig. 1 and Fig. 2) as shown below.

The effect of leaf extract against *Rhynchophorus ferrugineus* larvae was used to test the susceptibility of *R. ferrugineus* for a submission period of 24 hr. at concentrations of 2.8, 4.2, 6.0, 7.0 and 8.0 ppm. Results showed that percentage of mortalities were as foloows; 55, 65, 75, 80, and 85, respectively (Table 1 and Fig. 1). The concentrations reflected an LC₅₀ of 2.7 ppm, and LC₉₅ of 2.6 ppm. The lowest concentration (2.8 ppm) produced 55% mortality, whereas the highest concentration (8 ppm) reflected 95% mortality and the R-square was 0.79.

Also, the flower extract was used to test the susceptibility of *R*. *ferrugineus* larvae at the concentrations of 2.8, 4.2, 6.0, 7.0 and 8.0 ppm. Results showed that percentage of mortalities were as follows; 55, 70, 75, 80 and 85, respectively. The concentrations reflected an LC_{50} of 2.6 ppm, an LC_{95} of 9.6 ppm. The lowest concentration (2.8 ppm) produced 55% mortality, whereas the highest concentration (8 ppm) reflected 85% mortality and the R-square was 0.76.

In addition, the stem extract was used to test the susceptibility of *R. ferrugineus* larvae at the concentrations of 2.8, 4.2, 6.0, 7.0 and 8.0 ppm. Results showed that percentage of mortalities were as follows; 50, 55, 65, 75 and 85, respectively. The concentrations reflected an LC_{50} of 2.8 ppm, and LC_{95} of 8.9 ppm. The lowest concentration (2.8 ppm) produced 50% mortality, whereas the highest concentration (8 ppm) reflected 85% mortality and the R-square was 0.96.

Finally, the root extract was used to test the susceptibility of *R*. *ferrugineus* larvae at the concentrations of 2.8, 4.2, 6.0, 7.0 and 8.0 ppm. Results showed that percentage of mortalities were as follows; 35, 40, 45, 50 and 70, respectively (Table 2, Fig. 2). The concentrations reflected an LC_{50} of 7.00 ppm, and LC_{95} of 13 ppm. The lowest concentration (2.8 ppm) produced 35% mortality, whereas the highest concentration (8 ppm) reflected 70% mortality and the R-square was 0.96.

4. Discussion

In general, the current results of the leaf extracts showed relatively higher larvicidal potentiality against *R. ferrugineus* larvae, where root extract showed less potentiality than the other plant parts. Also, the relative efficiency of the plant parts extracts against *R. ferrugineus* larvae can be ordered for its stronger larvicidal effect as follows; leaf, flower, stem and then root, respectively.

Similar findings were noticed by Al-Barty and Hamza (2015) in a study of the titanium dioxide nanoparticles (TiO2NPS) made from *Moringa oleifera* extracts, was found to have larvicidal effect against the *R. ferrugineus* concluded the TiO2NPS potentiality to be used along with ectracts from *M. oleifera* against *R. ferrugineus* larvae. Plant extracts containing high concentrations of nicotine-rich compounds can rapidly knock down all tick species and stages. In order to link between nicotine and successful knockdown of ticks, pure alkaloid at high concentrations was tested and recorded in the tobacco extracts. In addition, Hosh et al, (2015) mentioned that

Table 1

Effect of leaf and flower extracts against R. ferrugineus larvae.

Conc. (ppm)	Log-Conc	Leaf extract		Flower extract	
		Probit	Mortality %	Probit	Mortality %
2.8	0.45	5.13	55.0	5.13	55.0
4.2	0.62	5.39	65.0	5.30	70.0
6.0	0.78	5.67	9975.090	5.47	75.0
7.0	0.85	5.84	80.0	5.78	80.0
8.0	0.9	6.04	85.0	6.12	85.0
R ²		0.79		0.76	
Slope		2.10		2.82	
x-coefficient		2.52		2.28	
LC ₅₀		2.7 ppm		2.6 ppm	
LC ₉₅		11 ppm		9.6 ppm	

Table 2

Effect of stem and root extracts against R. ferrugineus larvae.

Conc. (ppm)	Log-Conc	Stem extract		Root extract	
		Probit	Mortality %	Probit	Mortality %
2.8	0.45	5.00	50.0	4.51	35.0
4.2	0.62	5.13	55.0	4.83	40.0
6.0	0.78	5.39	65.0	4.91	45.0
7.0	0.85	5.67	75.0	5.00	50.0
8.0	0.9	6.04	85.0	5.41	70.0
R ²		0.96		0.76	
Slope		10.93		9.09	
x-coefficient		2.28		2.93	
LC ₅₀		2.8 ppm		7.0 ppm	
LC ₉₅		8.9 ppm		13 ppm	

Table 3

Relative efficiency of the N. glauca extracts on R. ferrugineus larvae according to the obtained values of LC_{50} and LC_{95} .

N. glauca extract	R. ferrugineus larvae		
	LC ₅₀	C ₉₅	
N. glauca leaf	2.6	9.6	
N. glauca flower	2.7	11	
N. glauca stem	2.8	8.9	
N. glauca root	7	13	



Fig. 1. Log-Probity curve of leaf and flower extracts against *R. ferrugineus* larvae.

many medicinal plants in the African folk medicine such as Gnidia glauca are famous for their pesticidal, insecticidal, molluscicidal and even homicidal activity for its use as arrow poisons.

The current findings showed remarkable potentiality of *N. glauca* extracts as a biological insecticide against *R. ferrugineus*. This could be explained as the *N. glauca* extracts contain a relatively high concentration the secondary metabolites compounds such as alkaloids. In light of this, the larvicidal effects of the *N. glauca* extracts may be attributed to the alkaloid anabasine, which has



Fig. 2. Log-Probity curve of stem and root extracts against R. ferrugineus larvae.

been confirmed previously in some phytochemical studies and bioassays as mentioned previously by (Keeler et al., 1981), (Mizrachi et al, 2000), (Panter et al, 2000) and (Weber, et al 2019). According to Zammit et al, (2014) the plant parts containing more of the poisonous anabasine complex are fruits and leaves, while roots, flowers and stems contain fewer amounts of it (Khafagy and Metwally, 1968). This result in agreement with the current findings as leaf extract showed the highest larvicidal effect against *R. ferrugineus* larvae while root extract showed the lowest effect. Finally, Baldwin and Callahan (1993) explained that *N.* glauca accumulate nicotine and anabasine in its different parts in order to defend itself against herbivores.

5. Conclusion

Although, *Nicotiana glauca* plant is toxic to humans and animals, using its poisonous extracts might be a better approach in pest management with less harm to both environment and human health. Therefore, the current study investigated the larvicidal effect of *N. glauca* aqueous extracts against the red palm weevil larvae (*Rhynchophorus ferrugineu*). It concluded that *N. glauca* extracts

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showed remarkable potentiality as insecticidal substances that can be used as an ecofriendly integrated approach for the management of *R. ferrugineus*. However, further investigation on identifying *N. glauca* active compounds and their mechanisms of action against *R. ferrugineus* is recommended.

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