INT. J. BIOL. BIOTECH., 19 (4): 511-515, 2022.

INSECTICIDAL EFFECT OF ETHANOLIC LEAF EXTRACT OF *CONOCARPUS LANCIFOLIUS* ENGL. AGAINST KHAPRA BEETLE

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ABSTRACT

The extract from the leaves of *Conocarpus lancifolius* Engl. was obtained and its toxic effect was assessed on the larvae of khapra beetle (*Trogoderma granarium* Everts). The leaves of *C. lancifolius* were extracted in ethanol. Four concentrations (2, 4, 6 and 8 µL mL^{-1} acetone) were prepared and applied through contact method. Toxicity was observed after 12, 24 and 36 h. All the concentrations showed toxic effects on the larvae of *T. granarium*. The highest larval mortality (38.34%) was observed at 8 µL mL^{-1} acetone concentration after 36 h. At the same concentration, the observed mortality was 31.67% and 21.67% after 24 h and 12 h, respectively. The least mortality (8.34%) was observed at 2 µL mL^{-1} acetone concentration after 12 h of exposure. The results indicated that *C. lancifolius* had potent toxic effect on *T. granrium* that increased by increasing the time of exposure and concentrations of the extract.

Keywords: *Conocarpus lancifolius,* Insecticidal effect, Khapra beetle, Leaf extract, Natural pesticides.

INTRODUCTION

Khapra beetle is a serious pest of stored grains (Golizadeh and Abedi, 2016). It is listed among the world's most invasive species. It was recognized by EPPO as an A2 quarantine organism (Honey *et al.,* 2017). It occurs on commodities which have been imported from the native countries and it can spread because of more use of cargo containers and can be rolled on and off on transport. These variables can make it prospective threat to the global food security. It is a major stored grain pest of wheat, maize and rice in Pakistan (Ahmad *et al.,* 2017). It can occur commonly in bins, farm houses, granaries, godowns and silos (Ahmedani *at el.,* 2007). The host range of khapra beetle includes rice, wheat, maize, sorghum, cotton, groundnut, millet, sesame, barley and cowpea (Singh *et al.,* 2017). It is a cosmopolitan and polyphagus insect (Musa and Dike, 2009). It can be of public health concern as the skin of larvae can cause dermatitis in humans. The hair present on the body of larvae are capable of causing allergies in human if swallowed. The larvae feed on grains and thus convert them to powdered material and in addition lay their feces in them. This reduces the grain quality as well as its nutritional value. Due to presence of this pest, the import of product is banned (Ahmedani *et al.,* 2007). The rice export to Mexico and United States of America was rejected between the year 2011-2013 due to the presence of khapra beetle and as a result, Pakistan had to endure a loss of 1000 million rupees (Honey *et al.,* 2017).

The first line of control for khapra beetle is fumigation with methyl bromide, but its identification as a major ozone depleting agent has led to its ban in various countries (Honey *et al.,* 2017). Insecticides like malathion, pyrethrins and chlorpyrifos are also commonly used for control of this pest (Honey *at el.,* 2017). The larvae are capable of hiding in cracks and crevices so their use have limitation as they are contact insecticides. Due to extensive use of insecticide, the insects have started to acquire resistance against them so their efficacy is declining. A physical method to control khapra beetle include modified temperature extremes. Exposing the pest to extremely high or low temperature can affect the physiology and mortality of pest. High temperature can affect physiology of grains which can reduce nutritional quality of grain, so it is more desirable to use cold temperature as compared to high temperature (Honey *at el.,* 2017). The effect of biological control agent including entomopathogens, predators and nematodes have also been studied against khapra beetle but they have not been commercially used (Honey *at el.,* 2017).

The research regarding the use of botanicals to control various pests and diseases including insects (Pavela, 2016), fungi (Javaid *et al.,* 2018; Khan *et al.,* 2020) and weeds (Javaid, 2010; Javaid *et al.,* 2020) is increasing these days. Botanical insecticides are eco-friendly due to their less persistency in the environment. The toxicity of botanical insecticides against non-target organisms had hardly been reported in the litérature. Synthetic chemical insecticides have high persistency and toxic effect on non-subjected organisms. In addition the phenomenon of insecticidal resistance is increasing day by day so scientists, farmers and consumers are more inclined towards use of botanical insecticides as alternatives (Hikal *at el*., 2017). The insecticidal effect of various plant extracts including garlic (*Allium sativum*), camphor (*Eucalyptus globulus*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sunflower (*Helianthus annuus*), onion (*Allium cepa*) and olive (*Olea europaea*) has been studies against khapra beetle and found effective (Younes *at el*., 2011).

Conocarpus lancifolius Engl. is a tree of family Combretaceae, indigenous to Somalia, Yemen and Djibouti. During the past few years, it has been extensively planted in different areas of Pakistan. Its insecticidal effect has not been extensively studied but its antibacterial and antifungal properties have been reported (Tougeer *at el*., 2014). In a study, the toxic effect of *C. lancifolius* was observed on green peach aphid (*Myzus persicae*). On tomato plant, 78% mortality of *M. persicae* was observed while on eggplant 79% mortality of the aphid was observed (Alyaseri *at el.*, 2020). It was hypothesized that if *C. lancifolius* has anti-bacterial and antifungal properties, it might have insecticidal effect against other pests such as khapra beetle. Therefore, this study was carried out to investigate the effect of ethanolic leaf extract of *C. lancifolius* against larvae of khapra beetle.

MATERIALS AND METHODS

The experiment was carried out in the Integrated Pest Management Laboratory, Department of Entomology, Faculty of Agricultural Sciences, University of the Punjab during 2022.

Maintenance of Culture

The culture of khapra beetle was obtained from University of Agriculture Faisalabad. Magnifying glass was used for the identification of egg, larvae and pupae of the beetle. A glass jar was filled with 100 g wheat, which contained all life stages of the beetle. To prevent an escape of beetle and to provide proper aeration, the open end of the jar was covered with mesh cloth with the help of rubber band. Temperature was maintained at 30 ºC ± 2 with the help of air conditioner. Humidity was maintained at 70% ± 5 with the help of humidifier. The photoperiod was 13 h light and 11 h dark.

Preparation of extract

The leaves of *C. lancifolius* were collected from Bahria Orchard, Lahore. The leaves were then washed with distilled water to remove any impurity including dirt, debris and microorganisms. The leaves were allowed to dry for 5 days. The leaves after drying were crushed with the help of pestle and mortar and then grinded by electric blender to fine powder. In a conical flask, 100 g powder and 500 mL of 95% ethanol was added. The flask was shaken continuously for 10-15 min. The plastic was tightly placed on the open end of flask with the help of rubber band. The apparatus was left for 2 days for proper extraction of compounds from the plant material. Thereafter, the extract was filtered through Whatmans filter paper. The extract was then transferred to glass tubes and stored in refrigerator at 4 ºC.

Application of extract

A dose of 2, 4, 6 and 8 µL was applied directly on Petri dishes with the help of micro-pipette and to spread the concentration evenly 1 mL acetone was applied. Three replicates were used with completely randomized design. The laboratory conditions were 30 ^{o}C ± 2 temperature and 70 ± 5% relative humidity. Twenty, 3rd instar larvae of khapra beetle were released in the Petri dishes with the help of forceps. Mesh cloth was placed on the open end of Petri dished with the help of rubber band to provide aeration. On a control only 1 mL of acetone was applied. Mortality rate was counted after 12, 24 and 36 h of exposure.

Statistical analysis

The data obtained were subjected to 2-way analysis of variance (ANOVA) to observe the effect of time and concentration on mortality. To compare the means of treatments, Turkeys HSD test was applied after ANOVA using Statistix 8.1 software.

RESULTS AND DISCUSSION

ANOVA clearly indicates that the effect of extract concentration (C), time intervals (T) and their interaction (C × T) was significant for mortality of khapra beetle (Table 1). Acetone was used for spreading of extract both in control and extract treatments. Its effect was very negligible. After 12 h of incubation, there was 0% mortality in control that raised to 1.66% and 5% after 24 and 36 h incubation, respectively. After 12 h incubation, 2, 4, 6 and 8 µL leaf extract treatments caused 8, 17, 18 and 22% mortality of the larvae, which was further increased to 17, 17, 28 and 32% after 24 h, and 22, 28, 35 and 38% after 36 h, respectively (Fig. 1).

Table 1. Two-way analysis of variance regarding percentage mortality of khapra beetle (*Trogoderma granarium*) due to different concentrations of *Conocarpus lancifolius*.

Source of variance	DF	SS	MS	F	P
Concentration (C)	4	4642	1161	161	0.000^{***}
Time (T)	2	1141	571	79	0.0000^{***}
C × T	8	264	33	4.58	0.010^{**}
Error	30	217	7.22		
Total	44	6264			

, * show significant different at P ≤ 0.01 and 0.001, respectively.

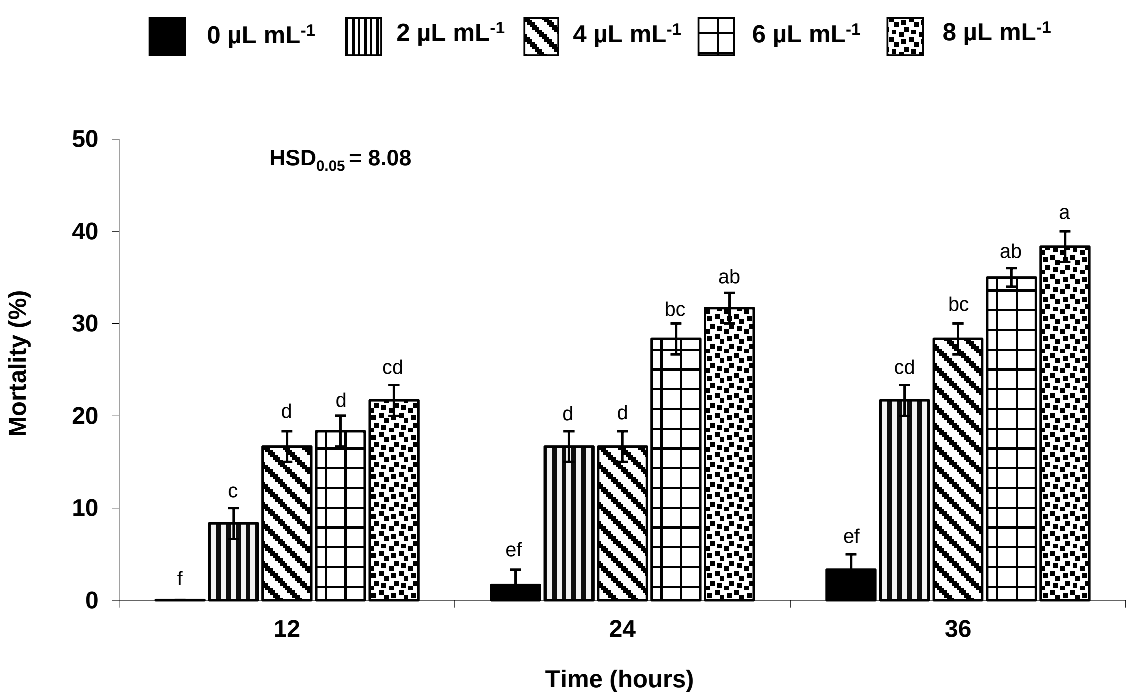

Fig. 1. Effect of different concentrations of ethanolic leaf extract of *Conocarpus lancifolius* on mortality of khapra beetle (*Trogoderma granarium*). Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by HSD Test.

Earlier, Osman *et al*. (2016) observed toxicity of *C. lancifolius* on *Rhysopertha dominca* (lesser grain borer). A 10% concentration of aqueous leaf extract of *C. lacifolius* caused 46.7% mortality while 10% concentration of ethanolic leaf extract caused 52% mortality after 72 h of exposure. The mortality reached up to 56.7% at 15% concentration of ethanolic leaf extract. Likewise, Salih (2016) reported toxic effect of *C. lancifolius* on *Tribolium castaneum*. The concentration of 30% caused 46% mortality in larvae and 52.7% in adult after 24 h of exposure. Jasman *et al.* (2019) studied the toxic effect of aqueous extract of *Conocarpus erectus* on *Bemisia tabaci* and *Myzus persicae*. On the adult and larvae of *M. persicae,* 34.92% and 36.67% mortality were observed while 50.67% and

62.0% mortality were observed on adult and larvae of *B. tabaci*. Rahema and Shoker (2020) analyzed the chemical constituents of aqueous extract of *C. lancifolius* and reported the presence of saponins, coumarins, phenols, alkaloids, flavonoids, glycoside and terpenes compounds, some of which could be responsible for insecticidal properties of the plant. Raza *et al*. (2016) reported the chemical composition of ethanolic extract of *C. lancifolius*. The major compounds determined through UHPLC-Q-TOF-MS/MS analysis were gallic acid, corilagin, galloyl-3-O-glucoside, kaempherol-3-O-rutinoside, terflavin B, ellagic acid derivative, caffeic acid derivative and isorhamnetin. Generally, many plants of Combretaceae family exhibited insecticidal effects against various insects including *Terminalia chebula* insecticidal effects on *Aedes aegypti* and *Culex quinquefasciatus* (Veni *et al*., 2017), Cocos nucifera on various stored product pests (Rani *et al.*, 2017) and *Combretum micronthum* on various pests (Hiremath *et al.*, 1995). The present study concludes that extract of *C. lancifolius* has insecticidal properties. Further studies are needed to find out which compounds of leaf extract of plant are responsible for the control of khapra beetle.

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(*Accepted for publication October 2022*)