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Euphorbia bupleuroides Desf. latex as biopesticide against the red flour beetle (*Tribolium castaneum* [Herbst, 1797]) and khapra beetle (*Trogoderma granarium* Everts, 1898)

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***Euphorbia bupleuroides* Desf. latex as biopesticide against the red flour beetle (*Tribolium castaneum* [Herbst, 1797]) and khapra beetle (*Trogoderma granarium* Everts, 1898)**

Abstract: Laboratory evaluation of *Euphorbia bupleuroides* latex as biopesticide against the red flour beetle (*Tribolium castaneum*) and khapra beetle (*Trogoderma granarium*) were evaluated at ambient temperature. The insecticidal activity of latex was determined by direct contact application. Different concentrations were prepared by dilution of 2.5, 5.0, 7.0 and 10.0 µl of latex into 0.1 ml acetone. 1 µl was pumped regularly in the thorax of different insects. The latex of *E. bupleuroides* showed insecticidal activity against *T. granarium* and *T. castaneum*. High levels of mortality were associated with the increase in the concentration and time of exposure as well. *T. granarium* adults are generally more prone to latex insecticidal effects than *T. castaneum* adults. After 6 days of exposure to *E. bupleuroides* latex, the LC_{50} recorded was 14.12 µl for *T. granarium* adults, and 14.7 µl for *T. castaneum*. LC_{90} numbers, on the other hand, were 38.8 µl for the former, and 51.44 µl for the latter.

Key words: *Euphorbia bupleuroides*; biopesticide; latex; *Tribolium castaneum*; *Trogoderma granarium*; mortality

Mleček prerastolikega mlečka (*Euphorbia bupleuroides* Desf.) kot bioinsekticid za zatiranje riževega mokarja (*Tribolium castaneum* [Herbst, 1797]) in indijskega žitnika (*Trogoderma granarium* Everts, 1898)

Izvleček: Laboratorijsko vrednotenje mlečka iz prerastolikega mlečka (*Euphorbia bupleuroides* Desf.) kot bioinsekticida za zatiranje riževega mokarja (*Tribolium castaneum* (Herbst, 1797)) in indijskega žitnika (*Trogoderma granarium* Everts,1898) je bilo izvedeno pri sobni temperaturi. Insekticidna aktivnost mlečka je bila določena z neposrednim nanosom. Različne koncentracije so bile pripravljene z razredčenjem 2,5; 5,0; 7,0 in 10,0 µl mlečka v 0,1 ml acetona. 1 µl raztopine je bil previdno vbrizgan v oprsje hroščev. Mleček prerastolikega mlečka je izkazal insekticidno delovanje na oba preučevana hrošča. Velika smrtnost hroščev je bila povezana s povečanimi koncentracijami in daljšim časom izpostavitve. Odrasli osebki indijskega žitnika so bili na splošno bolj dovzetni za strupeni učinek mlečka kot odrasli osebki riževega mokarja. Po šestih dnevih izpostavitve mlečku je bila LC_{50} za indijski žitnik 14,12 µl in 14,7 µl za riževega mokarja. LC_{90} vrednost je bila za prvega 38,8 µl in 51,44 µl za drugega.

Ključne besede: *Euphorbia bupleuroides*; bioinsekticid; mleček; *Tribolium castaneum*; *Trogoderma granarium*; smrtnost

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1 INTRODUCTION

Pest insects can potentially be carriers of pathogens, and are a substantial contributing source of allergens. That is because of their large cosmopolitan population and high numbers at homes and other buildings (Chang and Anh, 2002). Interest in developing safer alternatives to potentially replace toxic chemicals in pest control is increasingly growing. Bioactive substances and plant insecticides serve different functions in pest control: act as repellents, impact oviposition or feeding, disrupt development, or serve as pest insecticides (Isman, 2017, as cited in Bohinc et al. (2020).

The use of plant extracts is one of the most desirable pest control methods (Salvadores et al., 2007). Plants secondary metabolites are recognized for their crucial role in pest control due to the selective, biodegradable, non-toxic nature of their products, as well as possessing fewer harmful side-effects on non-targeted organisms and the environment (Wink, 1993). Approximately 250,000 species of plants on earth have been labelled as possessing compounds with insecticidal properties (Rafael, 2001).

Euphorbiaceae family is one of the largest and most diverse family in the plant kingdom; comprising of 7800 species in 300 genera (Webster, 1994). Diterpenoids and triterpenoids secondary metabolites are substantially present in *Euphorbia* species (Giner et al., 2000). They are endowed with striking biological anti-cancer qualities; for instance, they can serve antitumor purposes (Tanaka et al., 2000), anti-proliferative (Cateni et al., 2010), anti-oxidant and cytotoxic (Aslanturk et al., 2013), and modulators of multidrug resistance (Vasas et al., 2012).

Euphorbia bupleuroides Desf. is labelled as an herbaceous plant, characterized by plain and simple leaves. It is commonly found in mountain rock areas (Quezel and Santa, 1963), and is utilized in Algeria as an endemic medicinal plant in traditional medicine with varied uses ranging from the extirpation of thorns to the treatment of warts, as well as the use of the decoction of roots for anti-inflammatory purposes. Furthermore, two of the major chemical compounds of *E. bupleuroides* are diterpenoids and triterpenoids (Aichour et al., 2014). They are the most relevant in the insecticidal nature of *E. bupleuroides* and similar plants (Singh, 2012; Vimal and Das, 2014).

This study is conducted to assess the toxicity of *Euphorbia bupleuroides* latex against grain pests, red flour beetle (*Tribolium castaneum* [Herbst]) and khapra beetle (*Trogoderma granarium* Everts).

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

In April 2018, samples of *Euphorbia bupleuroides* were collected from their natural habitat of mountain rock areas around Tazoult, east of the city of Batna in the North East of Algeria (35° 28' 54" N, 6° 15' 39" E).

2.2 LATEX PREPARATION

The stems of the collected samples of *Euphorbia bupleuroides* were cut using a knife, allowing the latex to come out into a container. Latex was then collected in beakers, which were then corked tightly to prevent both evaporation and solidification. They were, after that, labeled and preserved in a refrigerator to maintain freshness. The whole procedure took approximately three days overall.

2.3 INSECTS

Cultures of the red flour beetle (*Tribolium castaneum*) and khapra beetle (*Trogoderma granarium*) were maintained at 27 °C and 65 % relative humidity (RH) on a wheat flour of a growth culture room in the dark. Adult insects, 1–7 days old, were used for bioassay.

2.4 BIOASSAY METHODS

2.4.1 Insecticidal Activity

The insecticidal activity of latex was determined by direct contact application. Different concentrations were prepared by dilution of 2.5, 5.0, 7.0, and 10.0 µl of latex into 0.1 ml acetone. For each preparation, 1 µl was pumped regularly in the thorax of different insects; 10 adult insects were enclosed in a Petri dish. Controls were treated similarly but exposed only to acetone. Each concentration and control were replicated four times. Mortality percentage was determined at 2, 4 and 6-days following treatment. Upon the observation of no leg or antennal movements were, insects were considered dead. The percentage of insect mortality was calculated using Abbott's correction formula for natural mortality in untreated controls (Abdel-Sattar et al.,2010).

2.5 STATISTICAL ANALYSIS

Probit analysis of concentration-mortality data was conducted to estimate the LC_{50}, LC_{90} values, their 95 % confidence intervals and related parameters (Finney, 1971). Probit analysis was fitted using the ''LC_probit" function in the ''ecotox" package in R (Robertson et al., 2007).

3 RESULTS AND DISCUSSION

The insecticidal activity of *E. bupleuroides* latex was tested against *T. granarium* and *T. castaneum* (Table 1). Data on the accumulative mortality of the two stored products insect species along 6 days of exposure to latex revealed that *E. bupleuroides* latex was significantly toxic for both species, though at different concentrations, all slope regressions were qualified significant ($p < 0.05$) (Table 1).

The mortality rates of *T. castaneum* are summarized in Figure 1. At a concentration of 5 µl, mortality rates remained negligible from day 2 up to day 6. However, after 6 days, mortality rates increased from 5 to 90 %. The higher the concentration, the less time is required to achieve high levels of toxicity. At 7.5 µl and 10 µl, 10 and 42.5 % mortality rates are observable after 2 days, 7.5 and 20 % after 4 days, and 57.5 to 90 % after 6 days. The highest concentration (10 µl) caused 42.5 % a mortality rate just after 2 days of exposure (Figure 1).

The results on Figure 2 demonstrate that the mortality rate of *T. granarium* adults varied with concentrations and time. In fact, after 6 days, the recorded mortality rate was 15, 22.5, and 40 % using 5.0, 7.5, and 10.0 µl concentrations respectively.

Lethal concentrations of latex were calculated after 2, 4, and 6 days of exposure for both species. Results showed that *T. granarium* adults are generally more sensitive to latex insecticidal effects than *T. castaneum* adults (Table 1). Evidently, after 6 days of exposure to *E. bupleuroides* latex, the LC_{50} recorded was 14.12 µl for *T. granarium* adults, and 14.7 µl for *T. castaneum*. LC_{90} numbers, on the other hand, were 38.8 µl for the former, and 51.44 µl for the latter (Table 1).

Mortality rates increased with rising concentration levels of *E. bupleuroides* latex. Additionally, mortality rates of *T. castaneum* and *T. granarium* differed in accordance with the different concentration levels of *E. bupleuroides* latex.

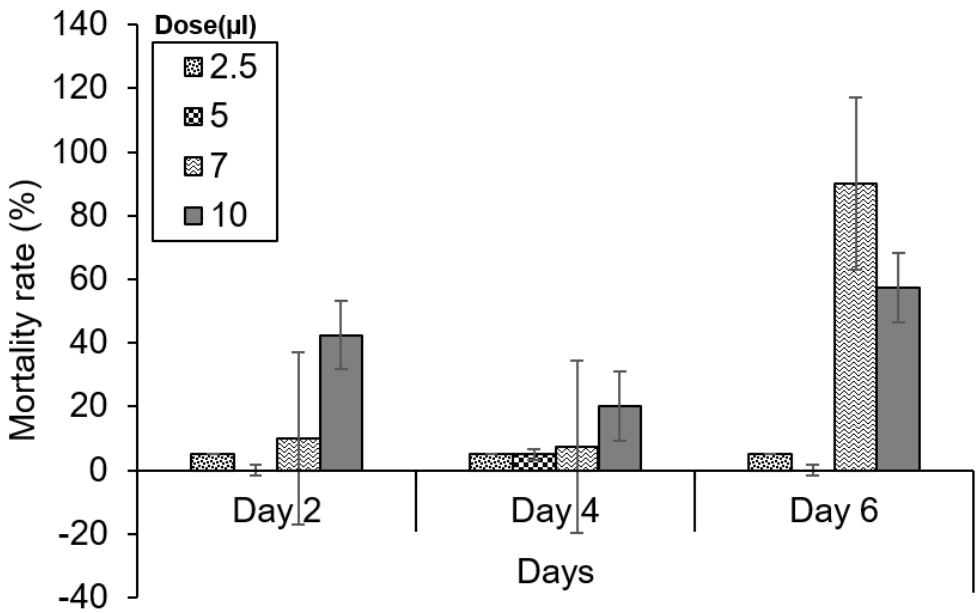

Figure 1: Mortality rates in T. castaneum treated with different concentrations of latex.

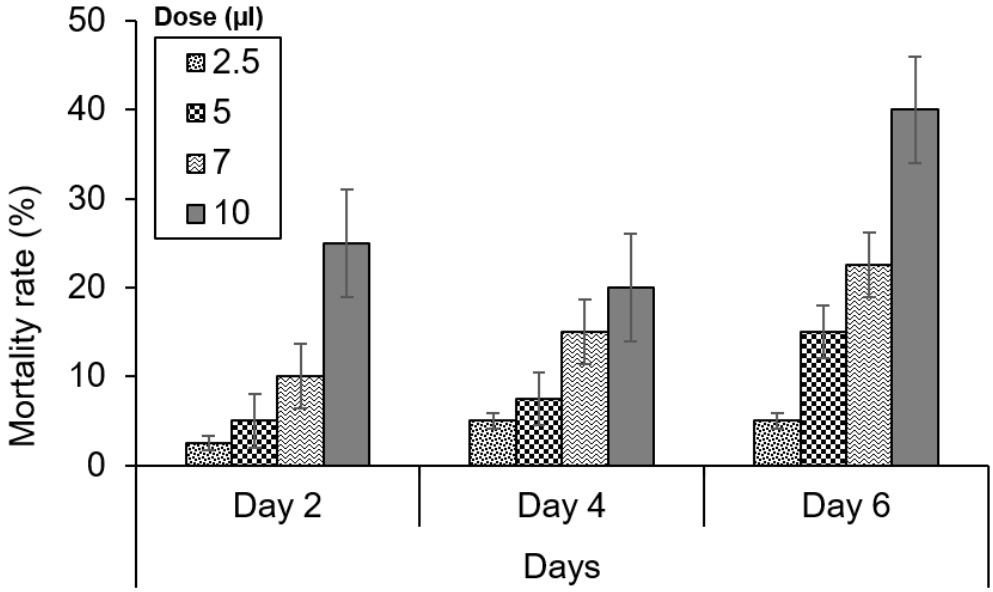

Figure 2: Mortality rates in *T. granarium* treated with different concentrations of latex.

Table 1: Latex toxicity on T. castaneum and T. granarium adults (LC_{50} and LC_{90}) after 2, 4, and 6 days.

Species	Assay time (days)	LC_{50} (µl)	95% LCL – UCL	LC_{90} (µl)	95% LCL – UCL	Slope ±SE	P
Tribolium castaneum	2	14.7	9.76 – 9.81E+3	38.8	17.02 – 1.93E+8	3.04 ± 0.860	0.0004
	4	56.03	17.98 – 5.23E-42	482.05	53.42 –3.77E-84	1.37 ± 0.715	0.049
	6	14.7	9.76 – 9.81E+3	38.8	17.02 – 1.93E+8	3.04 ± 0.860	0.0004
Trogoderma granarium	2	22.12	13.26 – 323.8	78.12	28.54 – 21222.5	2.33 ± 0.835	0.005
	4	40.74	16.10 – 3.613E+09	320.08	48.72 – 2.040E+19	1.43 ± 0.663	0.031
	6	14.12	9.44 – 105.5	51.44	21.14 – 9780.8	2.28 ± 0.625	0.0002

4 DISCUSSION

Different plants belonging to the Euphorbiaceae family have been studied all over the world for their toxic constituents. For instance, according to Govindarajan et al. (2008), Leaf extract of *Acalypha indica* L. exhibits larvicidal and ovicidal activities against malaria vector - *Anopheles stephensi* Liston, 1901. *Acalypha alnifolia* Klein ex Willd. extracted leaves demonstrated, in similar fashion to *Acalypha indica* L., larvicidal properties, but differed in having pupicidal effects—rather than ovicidal—against the same species (Murugan et al., 2011). De Silva et al. (2008) studied the insecticidal properties of *Euphorbia antiquorum* L. latex againt rice insect pests, whilst *Euphorbia fischeriana* Steud. had anti-feeding effects on stored-product insects according to Geng *et al.* (2011).

Euphorbia bupleuroides latex was tested several times against common species and pest insects that are widespread in houses, restaurants, and food stockages (Saito and Hama, 2000). For example, It was proven effective and toxtic against *Blattella germanica* Linnaeus, 1767 adults and larvae (Azoui et al.,2016) .

Vimal and Das (2014) confirmed that mortality rates increased with the increase in concentration of *Euphorbia antiquorum* L. latex extract. It was also found that latex was a strong pesticide against *Aedes aegypti* (Linnaeus and Hasselquist,1762) larvae, where LC_{50} val-

ue was 14.34 ml dl^{-1} after 24 hours, 10.70 ml dl^{-1} after 48 hours, 6.62 ml dl^{-1} after 72 hours of exposure.

The insecticidal effects of *Sebastiania corniculata* Müll. against *Laodelphax striatellus* (Fallén,1826), *Nilaparvata lugens* (Stål, 1854) and *Sogatella furcifera* (Horvath 1899), were evaluated by Lee et al. (2010). Results indicated that the chloroform fraction of *S. corniculata* possessed the highest potential for insecticidal activity against *L. striatellus* (DL50 = 1.09 µg/female), *N. lugens* (DL50 = .46 µg/female), and *S. furcifera* (DL50 = 2.32 µg/female). (LD stands for "Lethal Dose". LD_{50} is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test insects).

Mwine et al. (2010) examined larvicidal properties of *Euphorbia tirucalli* L. latex against larvae of *Anopheles* mosquitoes. Results showed that the latex made total mortality at the highest dilution used of 1:250 in 5 days. Plant latex comprises of a substantial mixture of proteins and specialized products that include alkaloids, terpenoids, cardenolides, and many other components, most of which are toxic against insects and pathogens (Agrawal and Konno, 2009; Hua et al., 2015; Huber et al., 2015; Konno, 2011; Konno et al., 2006).

Numerous ingredients have been isolated from the extracts of *Euphorbia* species (Jain et al., 2008). Al-Younis and Abdullah (2009) identified flavonoids and phenolic acids from several species of *Euphorbia* genus including *Euphorbia granulata* Forssk. and *Euphorbia heliscopia* L. Different triterpenoids and diterpenoids were isolated from various *Euphorbia* species (Sutthivaiyakit et al., 2000; Sun et al., 2011; Aichour et al., 2014). According to Lima et al (2006), terpenoids are the most significant element in the insecticidal property of several plant species.

5 CONCLUSION

This study confirms the success of latex as a biopesticide against certain stored products insect species; namely, *T. castaneum* and *T. granarium*. Hence, latex could serve as an alternative to synthetic insecticides for the protection of stored grain. Further studies and investigations are necessary to isolate the active insecticidal compounds of the latex and study the insecticidal effects of these compounds.

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