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Toxicity assessment of *Cedrus deodara* oil compared to carbosulfan for *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults

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Abstract. Specific compounds extracted from plants can control insect pests. The objective of this study was to evaluate the toxicity of deodar oil (phytopesticide) to adult mealworms *Tenebrio molitor* (Coleoptera: Tenebrionidae) compared with carbosulfan (synthetic insecticide), which exibits cholinesterase (ChE), glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) activities. The insecticides were applied through feeding, and the LC_{50} (lethal concentration) was calculated using the Finney method. The LC_{50} of deodar oil was higher than that of carbosulfan. The doses of both deodar oil and carbosulfan inhibited the ChE activity ($p > 0.05$) and enhanced the GPT and GOT activities ($p < 0.05$) in mealworm adults. Alterations in the activity of these biomarkers indicated that deodar oil could effectively control adult mealworms, being an environmentally low-impact method that can replace the use of chemical products.

Keywords: *Cedrus deodara*, cholinesterase (ChE), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), phytotoxicity, *Tenebrio molitor*.

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1. INTRODUCTION

Synthetic pesticides are effective against insects but pose a threat to human health. Chemical insecticides cause pollution, insect resistance, and negative impacts on beneficial insects and human health, besides contaminating the environment. These issues increase the importance of evaluating and using products that include eco-friendly compounds with insecticide properties, detected in 2400 plant species (Liu and Ho 1999; Abubakar et al. 2000; Tripathi et al. 2000; Umoetok 2000; Meera and Mann 2002; Kanvil et al. 2006).

The oil from the Deodar tree, *Cedrus deodara*, can repel and control livestock pests that cause foot infections for horses, cattle, and camels (Gamble 1902), and is toxic to stored product pests and *Musca domestica* (Diptera: Muscidae) (Singh et al. 1984; Singh and Rao 1985; Singh and Agarwal 1988; Singh et al. 1989). Moreover, this oil has antibacterial (Devmurari 2010) and antifungal properties (Mahalingam et al. 2011); its himachalene and atlantone fractions are larvicides (Chaudhary et al. 2011).

Carbosulfan inhibits cholinesterase (ChE) and prevents the acetylcholine breakdown into acetic acid and choline, resulting in abnormal neurotransmission (Namba et al. 1971; Menozzi et al. 2004). *Liposcelis bostrychophila* (Psocop: Liposcelididae) is sensitive to carbosulfan (Cheng et al. 2004); *Allium sativum*, *Azadirachta indica* and *C. deodara* oils are toxic to this insect (Rao et al. 2003). Bio-insecticides affect glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), which are important biomarkers to detect cell rupture and cellular impairment (Mead-Hala 2000; Malarvizhi et al. 2012).

Tenebrio molitor (Coleoptera: Tenebrionidae) is a pest of flour, grain, and other stored products (Ramos-Elorduy et al. 2002), and *C. deodara* oil was able to control the larvae and pupae of this insect in Pakistan (Buneri et al. 2018; Buneri et al. 2019).

C. deodara oil may decrease the activity of larval biochemical parameters (such as GPT and GOT), but there are no studies available regarding such an effect of *C. deodara* oil on mealworms. This research was conducted to evaluate the toxicity of the phytopesticide deodar oil, comparing it with the toxicity of the synthetic insecticide carbosulfan, by evaluating their LC_{50} values and effects on the ChE, GPT and GOT activities in *T. molitor* adults.

2. MATERIALS AND METHODS

2.1. Insect collection and rearing

Adult mealworms (*T. molitor*) used in this research were obtained from the Karachi Port Trust (KPT), Pakistan, and reared at a temperature of 25–30 °C and humidity of 70% in the Toxicology Laboratory at the Department of Zoology, University of Karachi, Pakistan. This insect was reared inside a deep, open bowl (depth 15 cm and width 30 cm) on about 1 kg of wheat bran and 1 kg of potatoes (Morales-Ramos et al. 2011).

2.2. Chemicals

Deodar oil was extracted by the traditional method (Khyber Pakhtunkhawa, Pakistan) using chips of *C. deodara* cut from the tree, washed, and dried under shade at 24 °C for one week, then placed in a kettle. The kettle was heated and about 200 mL of deodar oil (oozing drops) was collected in a separate container, cooled down and stored in a refrigerator at –20 °C until use. Deodar oil is composed of pentane, acetonitrile, himachalenes and atlantones (Chaudhary et al. 2011).

Carbosulfan (20 EC (emulsified concentration); STEDEC Technology Commercialization Corporation of Pakistan, Ministry of Science and Technology, Lahore) was purchased from an agricultural shop (Nawab Chemicals) in Karachi, Pakistan. It is a member of 1-benzofurans and a carbamate ester.

10% and 1% stock solutions of deodar oil and carbosulfan were prepared in methanol because this oil is insoluble in water. Serial dilutions of 8%, 4%, 2%, 1%, 0.5%, 0.03125%, 0.0625%, 0.125%, 0.25% and 0.5% of deodar oil and carbosulfan stock solutions were prepared. These concentrations were prepared from the stock solutions for each treatment with the help of Charles equation: $C_1V_1 = C_2V_2$, where C_1 represents the initial concentration of carbosulfan = 20%, deodar oil = 100%, C_2 denotes the final concentration, V_1 is the initial volume and V_2 the final volume.

The 1% carbosulfan stock solution was prepared based on the formula: $V_1 = C_2 \times V_2/C_1$, $V_1 = 1 \times 100/20$, i.e., V_1 = 5 mL, where C_1 = 20% (initial concentration), C_2 = 1% (desired concentration), V_2 = 100 mL (given volume), V_1 = (desired volume). 5 mL of carbosulfan was taken from the 20% solution and diluted in 95 mL of water to prepare the 1% stock solution (100 mL) of this chemical insecticide.

2.3. Treatment tests

The treatments were carried out with 8%, 4%, 2%, 1% and 0.5% of deodar oil and 0.03125%, 0.0625%, 0.125%, 0.25% and 0.5% of carbosulfan, mixing each concentration with bran in a plastic bowl (diameter 12 cm and depth 4 cm). Control and check groups were also used. Twenty adult beetles were exposed to each of these concentrations (n = 20 × 5 replicates) besides the control samples to determine whether the toxicity was due to

deodar oil or methanol. The check group was treated separately with methanol (bran + methanol) to determine whether the toxicity was due to deodar oil or methanol. The feed of the control group (bran + water) was without insecticides and methanol. The volume of insecticide per concentration to be fed to insects was kept constant, i.e., 2 mL/10 g of bran. Insect mortality was observed after 48 hours of the treatment.

2.4. Biochemical analysis (estimates of ChE, GPT and GOT activities)

The ChE activity was estimated by the Randox colorimetric method using the kit CE 190 (Randox Laboratories, United Kingdom), the GPT or ALT (alanine aminotransferase) activities were assessed by the colorimetric method with the help of the kit AL 146 (Reitman and Frankel 1957) and the GOT activity by using the kit D00678 (Hospi Lab Essentials, Pakistan).

2.5. Supernatant preparation

The LC_{50} of the insects was estimated per treatment. The insects were crushed, passed through a homogenizer and centrifugated at 3000 g. The organic extract obtained from this centrifugation was transferred to cuvettes and stored in a spectrophotometer model 721-2000 to estimate the enzyme activity (Buneri et al. 2018). The percent inhibition or enhancement of activity was calculated by the formula $(C_1–C_2)/C_1 \times 100\%$, where C_1 and C_2 are control and treated insect samples, respectively (Amin et al. 2022).

2.6. Statistical analysis

The mean percent value per parameter for the five replicates, standard deviation, standard error, and range were determined per each concentration of the insecticide and in the control group. The LC_{50} values were calculated (Finney 1971) using BioStat 2009 software, generated automatically by computer. The normal distribution of the data was checked using MS Excel. The one-way ANOVA was applied to evaluate the difference between the treated and control groups. The *p*-value lower than 0.05 ($p < 0.05$) was considered significant.

3. RESULTS

The serial dilutions of 8%, 4%, 2%, 1% and 0.5% of deodar oil caused 81%, 66%, 51%, 31% and 14% mortality, respectively, whereas 0.03125%, 0.0625%, 0.125%, 0.25% and 0.5% of carbosulfan caused 22%, 42%, 55%, 77% and 94% mortality in adult mealworms, respectively. There was no mortality in the control group, while 2% was determined in the check group. The LC_{50s} for deodar oil and carbosulfan were 2% and 0.09%, respectively (Figs 1, 2; Tables 1, 2).

The efficacy of the insecticide carbosulfan for *T. molitor* was about 21 times higher than that of deodar oil (Table 3).

Deodar oil and carbosulfan inhibited the ChE activity (125.12 U/L (unit per liter); $p > 0.05$)) by 5.88% in both treated *T. molitor* adults compared to the control group (Fig. 3; Table 4).

The GPT activity in *T. molitor* adults was 79.33 U/L and 95.33 U/L with deodar oil and carbosulfan, respectively. The activity of GPT was insignificantly ($p < 0.01$) increased in both deodar oil and carbosulfan treatments by 98.32% and 138.32%, respectively, compared to the control group (Table 5). The GPT percent activity is shown in (Fig. 4). The highest and lowest GPT percent activities were recorded as 238.32% and 198.32% in *T. molitor* adults treated with carbosulfan and deodar oil, respectively.

The GOT activities of *T. molitor* adults were 102.63 U/L and 167.15 U/L with deodar oil and carbosulfan, respectively. An increase ($p < 0.01$) of 7.36% and 74.81% was found with the deodar oil and carbosulfan, respectively (Table 5). The maximum and minimum percent

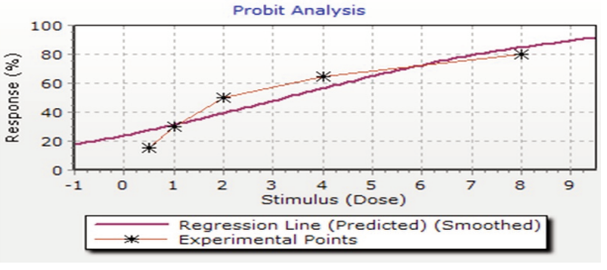

Fig. 1. Dose-response curve (generated automatically by BioStat 2009 software) of the mealworm (*T. molitor*) adults under the effect of deodar oil.

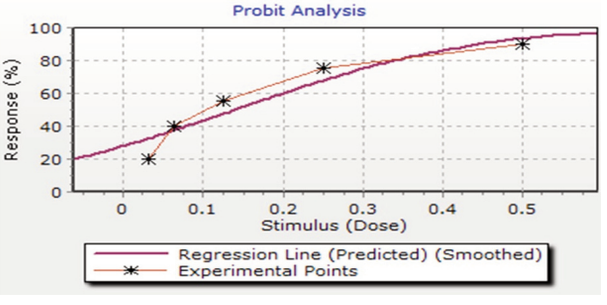

Fig. 2. Dose-response curve (generated automatically by BioStat 2009 software) of the mealworm (*T. molitor*) adults under the effect of carbosulfan.

Table 1. Toxicity evaluation of deodar oil and carbosulfan for the mealworm (*T. molitor*) adults

Deodar oil (Co)	MM	Me	SD	SE	Range
8	81	80	4.183	1.870	84.66–77.33
4	66	65	4.183	1.870	69.66–62.33
2	51	50	4.183	1.870	54.66–47.33
1	31	30	4.183	1.870	34.66–27.33
0.5	14	15	4.183	1.870	17.66–10.33
Check	2	0	2.738	1.224	4.4–0.400
Control	0	0	0	0	0.0–0.0
Carbosulfan (Co)	MM	Me	SD	SE	Range
0.5	94	95	4.183	1.870	97.66–90.33
0.25	77	75	2.738	1.224	79.40–74.59
0.125	55	55	5	2.236	59.38–50.61
0.0625	42	40	2.738	1.224	44.40–39.59
0.03125	22	20	2.738	1.224	24.40–19.59
Control	1	0	2.236	1.000	2.96–0.96

Co – concentration (%), MM – mean mortality (%), Me – median, SD – standard deviation, SE – standard error, Range – at 95% confidence interval

Table 2. Dose stimulus and percent mortality (computer generated) of the mealworm (*T. molitor)* adults treated with insecticides

Deodar	Carbosulfan	%Mortality
0.068494	0.004118	1
0.189135	0.010367	5
0.325103	0.016963	10
0.498847	0.025034	16
0.626567	0.030798	20
0.803942	0.03863	25
1.005604	0.047347	30
1.506137	0.068354	40
2.02101	0.096288	50
3.200849	0.135637	60
4.794053	0.19582	70
5.996601	0.240002	75
7.694174	0.30104	80
9.664117	0.370354	84
14.82892	0.546572	90
25.48931	0.894322	95
70.38464	2.251551	99
$LC_{50} \rightarrow 2.0\%$	$LC_{50} \rightarrow 0.09\%$	

Table 3. Comparative efficacy of the LC_{50} in the mealworm (*T. molitor*) adults

Treatment	LC_{50} (95 CI) %	Comparison: Deodar/Carbosulfan
Deodar	2.0	–
Carbosulfan	0.09	21

CI – confidence interval

Table 4. Effect of deodar oil and carbosulfan on the enzyme cholinesterase in the mealworm (*T. molitor*) adults

Insecticides	MA (U/L)	SD	SE	Range	PI	*p*-value
Control	132.94	13.544	7.829	148.285–117.594	0.0	
Deodar	125.12	13.544	7.829	140.465–109.774	5.88	0.38
Carbosulfan	125.12	13.544	7.829	140.465–109.774	5.88	
Check	132.94	13.544	7.829	148.285–117.594	0.0	

MA – mean activity, SD – standard deviation, SE – standard error, Range – at 95% confidence interval, PI – percent inhibition, *p*-value – <0.05 is considered significant

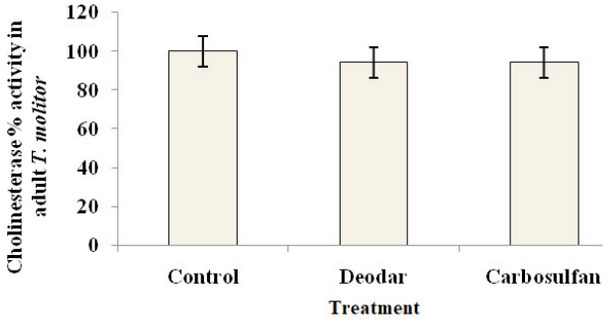

Fig. 3. ChE percent activity 94.12% for the mealworm (*T. molitor*) adults treated with deodar oil and carbosulfan.

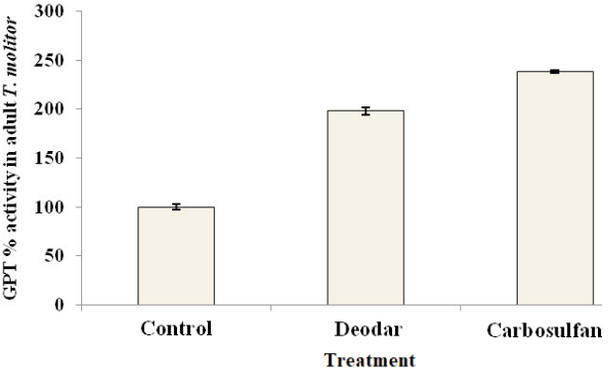

Fig. 4. Effect of deodar oil (2%) and carbosulfan (0.09%) on the GPT percent activity in the mealworm (*T. molitor*) adults.

activities were 174.84% and 107.36% for *T. molitor* adults treated with carbosulfan and deodar, respectively (Fig. 5).

4. DISCUSSION

This study fills in information gaps regarding the impact of deodar oil on the GPT and GOT activities of *T. molitor* as it is the first report on this insect, confirming the results of deodar oil on other insects, such as *Plutella xylostella*, *Callosobruchus chinensis*, *Anopheles stephensi* and *Sitophilus oryzae* (Singh et al. 1984; Singh and Rao 1985; Singh et al. 1989; Chaudhary et al. 2011).

Carbosulfan has higher toxicity to *T. molitor* than *C. deodara* oil, with 2% deodar oil killing 50% of mealworms adults, which conflicts with the previous reports for mealworm larvae with an LC_{50} of 3.41% (Buneri et al. 2019) – higher than the 2% obtained in the present study. This observation indicates that adult mealworms were more sensitive to deodar oil than their larvae. Kaur et al. (2021) observed fewer oviposition spots and no pupae in the pumpkin plant infested by fruit fly when the plant was treated with *C. deodara* oil. The LC_{50} of *C. deodara* essential oil for *T. molitor* adults was 2%, similar to the LC_{50} of 2.5% found for *Culex quinquefasciatus* (Makhaik et al. 2005).

Table 5. Effect of deodar oil and carbosulfan on the enzymes GPT and GOT of the mealworm (*T. molitor*) adults

Insecticides	MA (U/L)	SD	SE	Range	PE	*p*-value
GPT Control	40	5.196	3.000	45.880–34.119	0	0
Deodar	79.333	6.350	3.666	86.520–72.146	98.325	0.01
Carbosulfan	95.333	2.309	1.333	97.946–92.719	138.325	
Check	43	6.928	4.004	50.849–35.150	7.5	
GOT Control	95.599	2.488	1.759	99.0486–92.150	0	0
Deodar	102.637	4.147	2.932	108.386–96.888	7.361	0.01
Carbosulfan	167.152	4.147	2.932	172.901–161.403	74.846	
Check	97.359	3.317	2.346	101.957–92.760	1.840	

MA – mean activity, SD – standard deviation, SE – standard error, Range – at 95% confidence interval, PE – percent enhancement

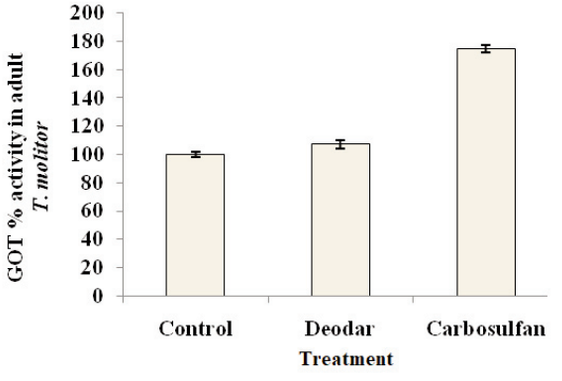

Fig. 5. Effect of deodar oil (2%) and carbosulfan (0.09%) on the GOT percent activity in the the mealworm (*T. molitor*) adults.

The high LC_{50} indicates that the efficacy of deodar oil is about 21 times lower than that of carbosulfan. The efficacy of deodar oil was low compared to the synthetic chemical, but research on this oil should continue because residues of plant origin products are low in storage facilities and insects develop resistance to chemical products (Jilani and Amir 1985). A possibility to overcome this low toxicity is to use higher doses for neem extract than those reported for conventional pesticides (Naqvi et al. 1989; Nurulain et al. 1994). On the other hand, *C. deodara* oil was efficient against the pests *Achatina fulica*, *Callosobruchus chinensis,* and *Culex quinquefasciatus* (Raguraman and Singh 1997; Rao and Singh 2002; Rahuman et al. 2009). The median lethal toxicity of plant extracts other than *C. deodara* oil to *T. molitor* has also been reported – the LC_{50} of *Allium sativum* oil being 0.203% for *T. molitor* adults in 48 hours (Plata Rudea et al. 2017) and that of *Nicotiana tabacum* 2.11% for the larvae of that insect in 24 hours (Sentosa et al. 2019). The additive effect of *Solanum nigrum* together with fenitrothion is toxic to the larvae of *T. molitor* (Spochacz et al. 2020). Our LC_{50} values are generally in a similar range to those previously reported and determined (Sentosa et al. 2019) and higher than those determined by some authors (Plata-Rueda et al. 2017), which may be due to differences in plant oil concentrations.

Carbosulfan reduced the population of *Thrips tabaci* (Zaman 1989) by a dose four times higher than that permitted against *Pachychila obtusecostata* (Tenebrionidae: Tenebrionidae) (Fegrouche et al. 2014).

Similar ChE inhibition and killing of *T. molitor* adults by deodar oil and carbosulfan corroborates the efficacy of the former product compared with the latter as a potent ChE inhibitor in mealworm pupae (Buneri et al. 2018). This agrees with the lower ChE activity for the pests of the snail *Lymnaea acuminate* and mosquito larvae treated with herbal molluscicides and *Sueda monoica* (Singh and Singh 2003; Yousuf et al. 2015). The inhibition of the ChE activity by 5.88% in adult mealworms by deodar oil was lower than that of azadirachtin oil by 11.1% in *M. domestica* (Rana et al. 2015). This higher inhibition may be due to differences in plant species, the synergistic effect of deodar, and species susceptibility. The mortality and ChE inhibition of *T. molitor* might be due to active compounds of *C. deodara*, which exhibit higher cholinesterase inhibition compared to the check and control groups.

The increase in the GPT and GOT activities in the supernatant of mealworms by deodar oil and carbosulfan agrees with the 21.3%:39.4% and 67.6%:136% higher LC_{50} values for aphid and whitefly with neem extract (Azab et al. 2011). The enzymatic activities of GPT and GOT in *T. molitor* treated with carbosulfan and deodar oil were higher than previously found in Sprague Dawley rats (Nwani et al. 2015), although in different organisms. Deodar oil and carbosulfan might have a strong negative impact on the mealworm fat body responsible for handling transferases and esterases in a similar manner to mammalian liver and enzymes, thus altering the response to the chemicals (Heong et al. 2011).

5. CONCLUSIONS

C. deodara oil was toxic to the mealworm (*T. molitor*) adults, acting as a potent ChE enzyme inhibitor. This oil has the potential to be a substitute for synthetic insecticides against insect pests as an environmentally friendly method. The LC_{50} of deodar oil was higher than that of carbosulfan; on the other hand, the doses of both chemicals inhibited the ChE activity ($p > 0.05$) and enhanced the activities of GPT and GOT ($p < 0.05$) in the mealworm adults. The LC_{50} of deodar oil was around 21 times lower than that of carbosulfan. The efficiency of deodar oil was very low compared to that of the synthetic chemical, but the toxicity of plant origin products is lower in stored grains. *C. deodara* oil can be used to protect grains against the mealworm (*T. molitor*) adults, reducing the use of conventional insecticides.

AUTHOR CONTRIBUTIONS

I. D. Buneri and M. Yousuf conceived research. I. D. Buneri, M. Yousuf and M. Attaullah conducted experiments. I. D. Buneri, J. C. Zanuncio, M. Ali, W. Khan, A. E. Krauklis, H. Ali, G. E.-S. Batiha, R. Khan, N. Ahmad, J. Burlakovs, R. H. Setyobudi, L. A. Shah, M. Zahoor, M. N. Azra and M. Naeem analysed data and conducted statistical analyses. I. D. Buneri, M. Yousuf, M. Attaullah, J. C. Zanuncio, A. E. Krauklis, M. Amin, H. A. Aouissi, N. Ahmad, J. Burlakovs, R. H. Setyobudi, L. A. Shah, M. Zahoor, M. N. Azra and M. Naeem wrote and approved the manuscript.

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Himaalaja seedri õli ja karbosulfaani toksilisuse hindamine ning mõju võrdlus täiskasvanud jahumardikatele (*Tenebrio molitor*)

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Himaalaja seedri (*Cedrus deodara*) õli on mürgine täiskasvanud jahumardikale (*Tenebrio molitor*) ja võib selle putuka puhul toimida ensüümi koliinesteraas (ChE) inhibiitorina. Himaalaja seedri õli võib asendada sünteetilisi insektitsiide, kuna see on keskkonnasõbralik meetod putukkahjurite vastu. Seedriõli 50% surmav kontsentratsioon jahumardikatele (LC_{50}) oli kõrgem kui karbosulfaanil. Nii seedriõli kui ka karbosulfaan pärssisid ChE aktiivsust vähesel määral ($p>0,05$) ja suurendasid oluliselt ensüümide glutamiinpürutransaminaas (GPT) ja glutamiinoksaloäädikhappetransaminaas (GOT) aktiivsust ($p<0,05$) jahumardika valmikutel. Seedriõli oli kahjurite vastu ligikaudu 21 korda väiksema mõjuga kui karbosulfaan. Sünteetiliste kemikaalidega võrreldes on seedriõli tõhusus väike, kuid taimset päritolu toodete kasutamisel tekib putukatel väiksem resistentsus insektitsiidide suhtes. Seedriõli saab jahumardikate kahjustuste vältimiseks kasutada tavapäraste insektitsiididega võrreldes väiksema resistentsuse riskiga.