

Research Article

Fumigation Toxicity of Essential Oil Monoterpenes to *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae)

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The fumigant toxicity of eight essential oil components, 1-8-cineole, carvacrol, eugenol, (-)-menthone, (-)-linalool, S-(-)-limonene, (-)- β -pinene, and (+)- α -pinene, was tested against the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae), at 0.25–60 $\mu\text{L/L}$ air doses. 1-8-Cineole, carvacrol, and eugenol caused complete adult mortality at 10 $\mu\text{L/L}$ air 24 h after treatment. 1-8-Cineole and carvacrol were the most toxic with LD₅₀ values of 0.24 and 0.6 $\mu\text{L/L}$ air at 24 h, respectively. (-)- β -Pinene and (+)- α -pinene were the least toxic with LD₅₀ values of 31 and 31.4 $\mu\text{L/L}$ air at 24 h, respectively. Toxicity was negatively correlated with vapor pressure. 1-8-Cineole and carvacrol caused 100% oviposition deterrence at all doses tested. Eugenol and (-)-menthone completely inhibited adult emergence. S-(-)-Limonene, (-)- β -pinene, and (+)- α -pinene were not effective at preventing oviposition or adult emergence, suggesting that a lethal dose of the three oil components would be necessary to control *C. maculatus* infestations.

1. Introduction

Cowpea, *Vigna unguiculata* (L.) (Walp.), is an important food legume for millions of people throughout the semi-arid regions of Africa, Asia, southern Europe, and North, Central, and South America [1]. The cowpea seed weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae), is the major pest of stored cowpea seed in the tropics and subtropics due to the favorable climatic conditions [2, 3]. The insect infests cowpeas in the field and the subsequent population buildup in storage can cause complete weight loss of stored cowpeas within six months if no prophylactic measures are put in place [4]. Life history and development of this insect on cowpeas have been described in early studies [2, 5].

Stored grain pest control has relied mainly on synthetic chemicals most of which pose serious dangers to life and the environment. Many organochlorine and organophosphate chemicals are already banned, while the use of some is being

restricted [6]. The use of methyl bromide, a major fumigant to control pests, is highly restricted [7]. Inhalation of phosphine gas, another grain fumigant, has been implicated in many human health problems [8]. Human health and environmental problems caused by synthetic pesticides, coupled with insects developing resistance to these chemicals, [9] have led to the need for alternative strategies that are ecofriendly and less prone to the development of resistance by pests [10]. Fumigation is still the most effective method of eliminating insect pests in stored products including cowpeas. The concern about the danger in the use of fumigants like methyl bromide and sulfuryl fluoride in pest control has shifted attention to evaluating essential oils as an effective and ecofriendly alternative [11].

An emerging pest control tactic for stored product pests is the use of essential oils. Essential oils are composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes obtained from plants through steam distillation [12].

Many studies have demonstrated contact and fumigant toxicity of plant essential oils and their components to several species of stored product insects at different life stages [13, 14]. Suthisut et al. [15] reported on the toxicity of essential oils of *Alpinia conchigera* (Griff), *Zingiber zerumbet* (L.) Roscoe ex Sm, *Curcuma zedoaria* (Christm.) Roscoe, and their components to *Sitophilus zeamais* (L.) and *Tribolium castaneum* (Herbst.). Various essential oils have also been tested against *C. maculatus* [16–18]. For example, *Hura crepitans* (L.) seed oil was reported as toxic to adult and immature stages of *C. maculatus* [16]. Similarly, oil of *Ziziphora clinopodioides* (Boiss) caused significant adult and egg mortality [17]. The majority of the above studies on the toxicity of essential oils against *C. maculatus* tested crude essential oils rather than individual synthetic compounds, making it difficult to attribute toxicity or oviposition deterrence to particular components. Furthermore, most of the studies did not determine the fate of eggs laid during fumigation (i.e., larval development and adult emergence from eggs laid during fumigation with the essential oils).

The objective of this study was to test the fumigant toxicity of some selected essential oil components at low concentrations against *C. maculatus*. The essential oil components tested in this study were chosen because they are major constituents of essential oils that have been found to have high pesticidal properties against store product pests, and the pure forms have also been reported toxic to some stored grain pests [12, 19–21]. Many essential oil components are known neurotoxins [20, 22–24]. We hypothesized that fumigation with low concentrations of the essential oil components will affect the nervous system of *C. maculatus* and thus prevent egg laying on stored seeds. A second hypothesis was that the efficacy of the essential oil components as fumigants is related to their physical properties. First, the fumigant toxicity of the oil components to adult *C. maculatus* was determined in fumigation chambers. The ability of females to lay viable eggs during fumigation was then assessed using the number of eggs laid. The fate of the eggs laid during fumigation was also determined by recording adult emergence. Finally, toxicity of the essential oil components was correlated with some physical properties of the oil components to identify the factors that best predict toxicity.

2. Materials and Methods

Eight essential oil components, 1-8-cineole, carvacrol, eugenol, (–)-menthone, (–)-linalool, S-(–)-limonene, (–)- β -pinene, and (+)- α -pinene, obtained from Sigma-Aldrich (St. Louis, MO) were tested in the study. The purity of the oil components ranged between 95 and 99%.

2.1. Cowpea Seed and Insect Culture. Cowpea (cultivar California black-eye) seeds were purchased from a local grocery store in Auburn, AL, USA. The seeds were kept in polythene bags, sealed and kept in a freezer maintained at -20°C for 72 hours until they were used for insect cultures or the bioassays [25].

A fresh culture of *C. maculatus* was started from a colony that had been maintained in the laboratory for over 50

generations by placing 25 pairs of 2-day-old male and female beetles in 1-L wide-mouthed glass Mason jars containing 100 g of cowpea seeds. The jars were placed in a rearing chamber maintained at $28 \pm 2^{\circ}\text{C}$, $70 \pm 5\%$ r.h, and 12:12 h photoperiod [16, 26]. The beetles were sexed using the keys described by Rees [27]. Female beetles were allowed to lay eggs on the seeds for 24 hours after which they were removed. The seeds containing eggs were kept in a rearing chamber until adult emergence.

2.2. Fumigation with Essential Oil Components. The fumigant toxicity of eight essential oil components at 0.25, 5, 10, 20, 40, and $60 \mu\text{L/L}$ was investigated by exposing five pairs of 1-day-old adult male and female *C. maculatus* to vapors from the oil components in 1 L wide-mouthed glass Mason jars containing 20 g of cowpea seeds. Jars without essential oil components served as the controls. Each treatment was replicated four times. The fumigation lasted for 24 hours at abovementioned environmental conditions. Data on adult mortality was recorded as a percentage of the total number of the beetles fumigated.

Data were arcsine transformed and analyzed using one-way analysis of variance (ANOVA). Tukey's HSD test was used to separate the means. Regression analysis was used to estimate LD_{50} in the adult mortality tests [28]. Stepwise multiple linear regression was used to reveal the physical property of the oil components that predicts LD_{50} .

To determine the effects of fumigant toxicity of the essential oil components on oviposition deterrence, the number of eggs on all cowpea seeds in each replicate was recorded after fumigation test. The egg-bearing seeds were returned back to the jars and placed in the rearing chamber undisturbed for four weeks after which they were examined for adult emergence. Adult emergence was monitored daily for 10 days starting from 30 days after treatment. Data on adult emergence were used to determine the fate of the eggs laid during fumigation. Logarithmic transformation was performed on oviposition deterrence and adult emergence data. ANOVA and Tukey's HSD test were used to compare the number of eggs laid and number of adults that emerged. All statistics were done using SPSS at $\alpha = 0.05$.

3. Results

3.1. Fumigant Toxicity to Adults. No mortality was observed in untreated controls during this study. The effect of the tested eight essential oil components on the survival of adult *C. maculatus* was concentration dependent. Three essential oil components, 1-8-cineole, carvacrol, and eugenol, caused $\geq 90\%$ mortality to adult beetles at doses of as low as $5 \mu\text{L/L}$ of air within 24 hours. (–)-Menthone, (–)-linalool, S-(–)-limonene, and the two structural isomers of pinene also caused 100% mortality to adult beetles, but at significantly greater doses ($20 \mu\text{L/L}$ and above) (Figure 1).

The LD_{50} values obtained in this study (Figure 2) were step-wisely regressed with the density, boiling point, and vapor pressure of the oil components. The analysis revealed vapor pressure as the physical property of the essential oil components that best predicts the LD_{50} ($F_{1,30} = 72.57$,

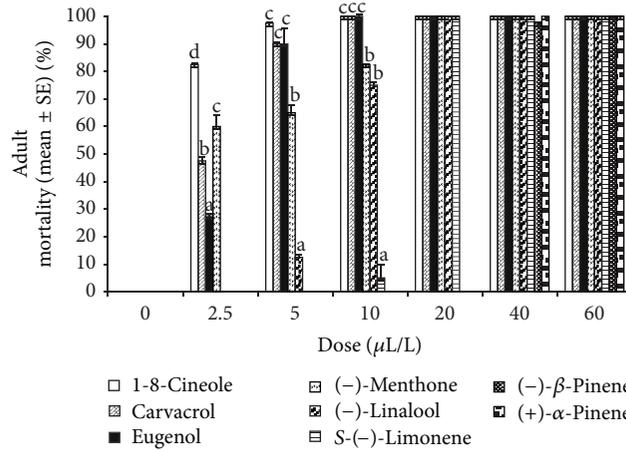


FIGURE 1: Mortality of adult *C. maculatus* exposed to eight essential oil monoterpenes.

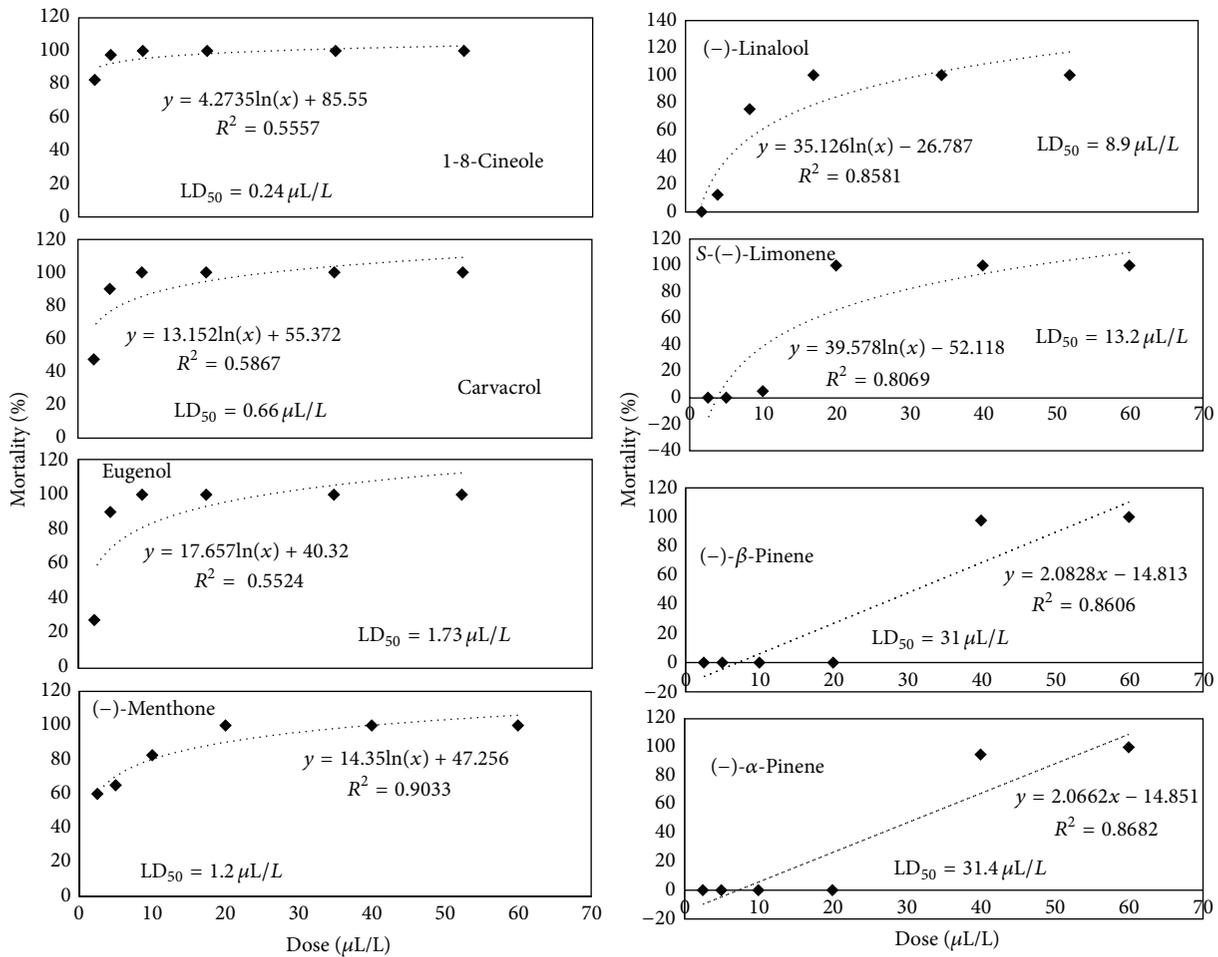


FIGURE 2: Dose-response curves of eight monoterpenes tested against *C. maculatus*.

$P = 0.001$) and accounted for over 70% of the variances. In general, toxicity was negatively correlated with vapor pressure (Figure 3). Based on the regression analysis, the predicted LD_{50} of essential oils can be calculated as follows:

$$\text{Predicted } LC_{50} = 13.044 \times \text{vapor pressure} + 3.081. \quad (1)$$

3.2. Oviposition Deterrence and Adult Emergence. 1-8-Cineole and carvacrol completely deterred oviposition at all doses tested (Figure 4). Numbers of eggs laid decreased with increasing dose for eugenol, (-)-menthone (-)-linalool, S(-)-limonene, and the two structural isomers of pinene. For example, eugenol-treated seeds had an average of 18.25

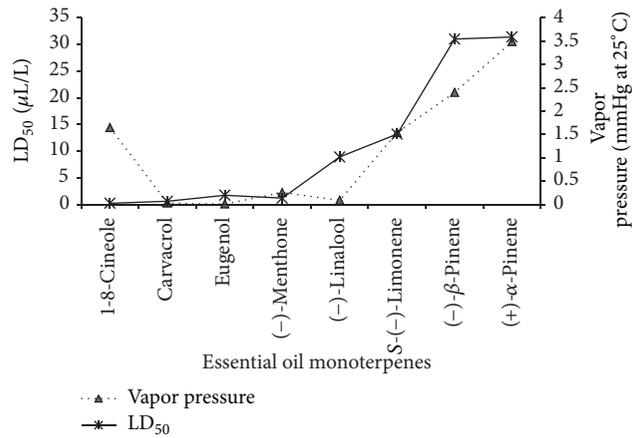


FIGURE 3: Relationship between LD₅₀ and vapor pressure of eight essential oil monoterpenes tested against *C. maculatus*.

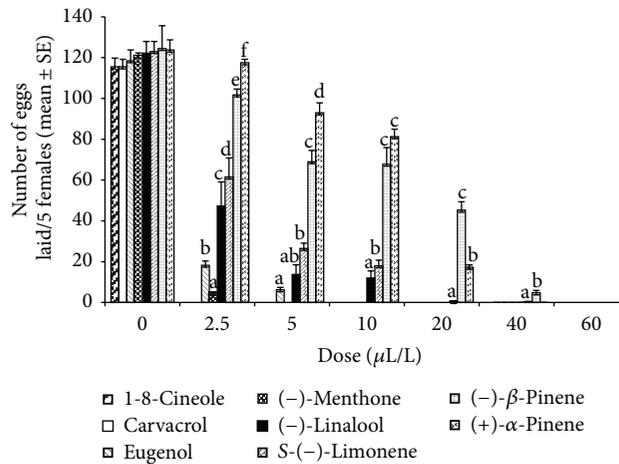


FIGURE 4: Number of eggs laid by *C. maculatus* during fumigation with essential oil monoterpenes. ANOVA and Tukey's HSD compared monoterpenes at each dose tested.

and 6.25 eggs at 2.5 and 5.0 µL/L, respectively. The two isomers of pinene had significantly higher numbers of eggs compared to (-)-linalool and S(-)-limonene. All eight essential oil components caused 100% oviposition deterrence at the highest dose tested (60 µL/L) (Figure 4).

Among the oil treatments which did not completely prevent oviposition, eugenol and (-)-menthone completely inhibited adult emergence at all doses tested (Figure 5). Adult emergence decreased with increasing dose of (-)-linalool, S(-)-limonene, and the two pinene isomers (Figure 5). Adult emergence in seeds treated with (+)-α-pinene and (-)-β-pinene was significantly ($P = 0.001$) higher than in linalool and S(-)-limonene, at 0.25–20 µL/L air (Figure 5).

4. Discussion

4.1. Fumigant Toxicity to Adults. The results of this study demonstrated fumigant toxicity of some essential oil components to *C. maculatus*. Three compounds, 1-8-cineole, carvacrol, and eugenol, caused almost 100% adult mortality at doses as low as 5 µL/L air. Similar levels of adult beetle mortality were achieved with the remaining five components,

(-)-linalool, S(-)-limonene, (-)-menthone, and the two structural isomers of pinene, but only at relatively greater concentrations (≥ 10 µL/L air).

Previous studies have reported on the toxicity of essential oils to *C. maculatus* and other stored product insect pests [21, 29–31]. The toxicity of essential oils tested was attributed to the components of the oils [20, 32]. Fahimeh et al. [29] reported toxicity of essential oil of *Zataria multiflora* (Boiss) (Lamiaceae) to *C. maculatus* at doses as low as 9 µL/L air 24 hours after treatment. It is worth mentioning that *Zataria multiflora* is a major source of carvacrol, which was also toxic to adult *C. maculatus* causing 100% mortality at 10 µL/L air in the present study. However, our LD₅₀ for carvacrol (0.6 µL/L air) (Figure 2) was about one-fourteenth of the LD₅₀ value (8.81 µL/L air) reported by Fahimeh et al. [29]. Sharifian et al. [33] reported that oil extracts of Mugwort (*Artemisia vulgaris* L.), a major source of (+)-α-pinene, were effective against *C. maculatus*. The estimated LD₅₀ of 53 µL/L air at 24 hours was greater than the LD₅₀ of 31.4 µL/L air at 24 hours obtained in our study using the neat form of (+)-α-pinene. Arezo et al. [34] estimated LD₅₀ of 24 µL/L air for (-)-linalool at 24 hours which was almost three times greater than the LD₅₀ value of

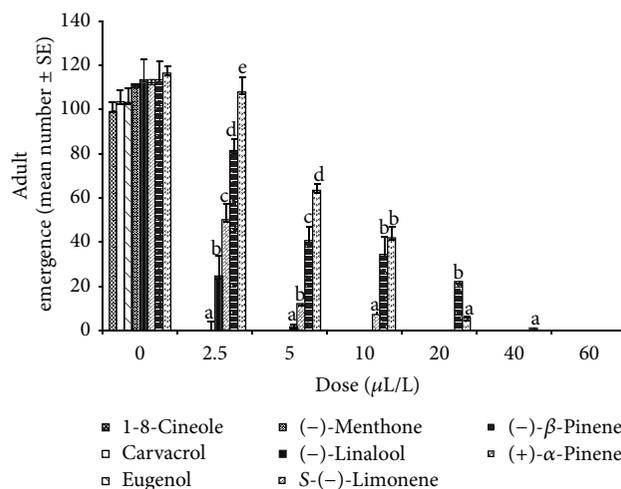


FIGURE 5: Number of adult *C. maculatus* that emerged from eggs laid during fumigation with essential oil monoterpenes. ANOVA and Tukey's HSD test compared monoterpenes at each dose tested.

8.9 $\mu\text{L/L}$ air obtained for (-)-linalool in this present study. Similarly, the 13.2 $\mu\text{L/L}$ air we obtained for S-(-)-limonene was eight times less than the LD_{50} value of 106 $\mu\text{L/L}$ air obtained by Ramin and Yunes [31] with essential oils from the peels of *Citrus sinensis* (L.). Synthetic (pure) monoterpenes were tested in the present study and that may explain the much lower LD_{50} values compared to that reported in the earlier studies with whole essential oils. Furthermore, it is plausible that other components of essential oils may have interacted to reduce efficacy of the monoterpenes in the whole oils tested.

Earlier studies have also revealed toxicities of the monoterpenes we tested in this study to other stored grain pests [35–38]. Carvacrol and 1-8-cineole were found toxic to *Tenebrio molitor* (F.) at LD_{50} of 5.52 and 5.71 μL , respectively [36]. Limonene had varied level of toxicity to *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), and *Tribolium castaneum* (Herbst.) [35]. Ogendo et al. [37] reported that mortalities of *S. oryzae*, *R. dominica*, *T. castaneum*, *Oryzaephilus surinamensis* (L.), and *Callosobruchus chinensis* (F.) with α - and β -pinenes ranged between 7.3–100% and 32.5–100%, respectively. (-)-Linalool and (-)-menthone were also found to cause 100% mortality of *S. zeamais* (L.) at 30 $\mu\text{L}/\mu\text{g}$ [38]. Source and purity may affect bioactivity of essential oils and the monoterpenes, and also age, size, sex, insect order, and species influence insects' susceptibility to chemicals [34]. Any of the above-mentioned factors or a combination of two or more of these factors may have accounted for the differences between the results obtained in the present study and the previous related studies.

4.2. Oviposition Deterrence and Adult Emergence. Two of the eight treatments, 1-8-cineole and carvacrol, completely deterred oviposition. (-)-Menthone and eugenol treated seeds had significantly fewer eggs while seeds treated with (+)- α -pinene and (-)- β -pinene had the highest numbers of eggs. Among the six treatments which did not cause complete

prevention of oviposition, eugenol and (-)-menthone completely inhibited adult emergence. The two pinene isomers had greater numbers of adult emergence. Previous related studies have tested the toxicity of crude essential oils to eggs of *C. maculatus* laid during fumigation [39, 40], but we are not aware of any studies that specifically tested synthetic (pure) essential oil components. Eman and Abass [40], in their study of efficacy of five essential oils against *C. maculatus*, reported significant reduction in oviposition with 1% acetone solution of *Mentha rotundifolia* (L.) and *Mentha pulegium* (L.). *M. rotundifolia* oil contains a low percentage (8.5%) of 1-8-cineole, while *M. pulegium* oil has 0.05, 0.2, and 0.4% of (+)- α -pinene, (-)- β -pinene, and S-(-)-limonene, respectively. Interestingly, S-(-)-limonene and the two isomers of pinene were not effective in the present study as oviposition deterrent or in preventing adult emergence even at high doses. Oil of *Elettaria cardamomum* (L.), a major source of 1-8-cineole (55.7%), also reduced oviposition by *C. maculatus* [40]. Other components of the oil may have interacted to lower the efficacy of 1-8-cineole content. Essential oils and their components possess low or no toxicity against vertebrates [20, 41, 42]. Acute toxicity (LD_{50}) of 1-8-cineole (2,480 mg/kg BW), eugenol (2,680 mg/kg BW), and linalool (>1000 mg/kg BW) in rats is far less than that of some approved plant-based pesticides like pyrethrins (300–350 mg/kg BW) and pyrethrum (1500 mg/kg BW) [20, 32, 43]. Besides, they are easily biodegradable; hence, the use of these oil components to protect cowpea seeds may not constitute problems to man and environment.

In summary, our results showed that 1-8-cineole, (-)-menthone, carvacrol, and eugenol are highly toxic to adult and egg stages of *C. maculatus*, even at low concentrations. Vapor pressure was the best predictor of toxicity of the essential oil components. While 1-8-cineole and carvacrol completely prevented oviposition, some eggs were laid on seeds treated with eugenol and (-)-menthone but the eggs did not develop to adults. These suggest that 1-8-cineole, carvacrol, eugenol, or (-)-menthone can be used to prevent

population build-up of *C. maculatus* in stored cowpea. The results also showed that fumigation with a single lethal dose or repeated treatment with (-)-linalool, S(-)-limonene, (+)- α -pinene, and (-)- β -pinene may be necessary for effective control of *C. maculatus* in stored cowpea. Essential oil components may also be combined with modified atmosphere or other tactics such as Purdue Improved Bag to achieve effective protection of stored cowpea.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publishing of this paper.

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