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CONTENTS

Fumigant toxicity of alpha-pinene, beta-pinene, eucalyptol, linalool and sabinene against Rice Weevil, *Sitophilus oryzae* (L.)

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ABSTRACT: Fumigant toxicity of alpha-pinene, beta-pinene, eucalyptol, linalool and sabinene was studied under laboratory condition against Rice Weevil, *Sitophilus oryzae* (L.) at 100,200,300,400 and 500µl/l air. The mortality of adult insects was recorded at 6, 12, 24, 48, 72 and 96 h after fumigation. The mortality of insects increased with increase in concentration of all the test chemicals and exposure time. Less than 50 per cent mortality was recorded after 6h fumigation in all the treatments even at highest dose. Exposure of insects to 500µl/l air eucalyptol, alpha-pinene and beta-pinene resulted in 96.7, 93.3 and 66.7 per cent mortality, respectively, after 12 h which increased to 100 per cent after 24h. As compared to aforementioned treatments, low toxicity was recorded in case of sabinene and linalool which showed 73.3 and 30.0 per cent mortality after 12h which increased to 90.0 and 90.00 per cent, respectively, after 24h. After 48, 72 and 96h fumigation all the treatments showed 100 per cent mortality at all the concentrations except 100 µl/l air. The present study revealed that alpha-pinene, beta-pinene, eucalyptol, linalool and sabinene were toxic to adults of *S.oryzae*, however, remarkable difference was noticed in their relative efficacy against this insect. Eucalyptol which killed all the insects at 200µl/l air after 24h exposure may be adjudged as most toxic followed by beta pine and alpha pinene which exhibited such high level of mortality at 300 and 400µl/l air during the same exposure. With high mortality at 100µl/l air after 24, 48, 72 and 96h exposure, linalool may be treated as more toxic as compared to sabinene. It may be concluded that all the tested monoterpenes are effective against *S. oryzae* and the essential oils having these chemicals may be used in the future for the fumigation of grain for its eco-friendly management.

Key words: Alpha-pinene, beta-pinene, eucalyptol, fumigant toxicity, linalool, sabinene, *Sitophilus oryzae,* wheat

Essential oil present in several plants are known to exhibit fumigant toxicity against many stored grain insect pests (Rajendran and Sriranjini, 2008; Koul *et al*., 2008; Kumar and Tiwari, 2018a; 2018b). Alpha-pinene, beta-pinene, eucalyptol, linalool and sabinene are found in essential oils of *Eucalyptus floribunda* (Myrtaceae), *Ocimum basilicum* (Lamiaceae), *Mentha piperita L.* (Lamiaceae), *Lavandula angustifolia* (Lamiaceae), *Citrus sinensis* (Rutaceae), *Artemisia dracunculus* (Asteraceae) etc. (Parsia *et al*., 2016; Germinara *et al*., 2017; Oboh *et al.,* 2017; Wahba and Attia, 2019; Gokturk *et al.,* 2020; Teke and Mutlu, 2020) which are toxic to insect pests of stored grain (Lee *et al*., 2002; Lee *et al*., 2003).

L-menthol has been found to exhibit vapor toxicity against *Callosobruchus maculates*(F.), *Rhyzopertha dominica* (F.), *S. oryzae* and *Tribolium castaneum* (Herbst.), having LC_{99}values (mg/liter space) 8.4, 7.9, 7.9, 28.1, respectively (Aggarwal *et al*., 2001). Menthol and L-menthone have also been reported to exhibit fumigant toxicity against *T. castaneum*, *Lasioderma serricorne* and *Liposcelis bostrychophila* adults (Pang *et al*., 2020). Exposure of *Tribolium confusum* Jacquelin du Valand *Ephestia kuehniella* (Zeller) adults to carvacrol resulted in more than 90 per cent mortality in both insects at 46.2 mg/l air within an exposure of 24–96 h but the larvae of *E. kuehniella* required a higher dose of 184.8 mg/l air. Another monoterpene γ-terpinene caused 99 per cent mortality in *T. confusum* and *E. kuehniella* after 26.4–57.5 h. The larva of *E. kuehniella* was reported to be the most tolerant insect stage (Erler, 2005). Monoterpene 1,8-cineole showed potential fumigant toxicity at 47.0, 18.8 and 21.6 µl/l air for 95 per cent kill of *S. oryzae*, *T. castaneum* and *R. dominica*, respectively (Lee *et al*., 2003) while it exhibited LC_{50} values at 14.19 and 17.16 mg/l, respectively, against former two insects (Abdelgaleil *et al*., 2009). In another study conducted against *T. castaneum* 1,8-cineole was the most toxic fumigant (LC_{50} = 7.4 µl/l air) followed by menthone (LC_{50} = 8.5 µl/l air) and p-cymene (LC_{50} = 11.4 µl/l air) (Lee *at al*., 2002). Ajayi *et al*. (2014) reported that 1,8-cineole, carvacrol, and eugenol caused total adult mortality of *C. maculatus* at minimum 10 µl/l air after 24 h of fumigation with LC_{50} values of 0.24 and 0.6 µl/l air at 24 h, respectively. Allyl isothiocyanate was reported to be toxic to *S. oryzae* with LC_{95} 10.8 µl/land LC_{50} 6.4µl/l (Cardiet *et al*., 2012). Sriti Eljazi *et al*. (2017) evaluated the fumigant toxicity of linalool against *T. castaneum*, *S. oryzae*, and *L. serricorne* (F.) and observed LC_{50} and LC_{95} at 90.89, 363.60; 172.37, 1402.26; and 31.79, 163.06 µl/l air, respectively, whereas linalool was found to be more toxic to *L. serricorne*. Cao *et al*. (2018) studied the fumigant

toxicity of linalool, beta-pinene, 3-carene, caryophyllene oxide and beta-caryophyllene against *T. castaneum*, *L. serricorne* and *L.bostrychophila* Badonnel and observed LC_{50}(mg/l air)values11.60, 27.25 and 0.82 for linalool, 3-carene and linalool against*T. castaneum, L. serricorne* and *L. bostrychophila,* respectively, after 24h exposure.

Review of literature indicates that some studies have been made to investigate the fumigant toxicity of monoterpenes against *S.oryzae.* Lee *et al.* (2004) reported that 95.3, 94.1 and 99.6 per cent reduction in emergence of adults of *S.oryzae*, respectively, was observed when egg, larvae and pupae of this insect were exposed to 1,8-cineole at 200 µl/l of air for 7 days. Alpha-Pinene was found to be toxic to *S. oryzae*after 24h exposure during which it killed 50 per cent population at 1.98 µl/cm^3 (Chaubey, 2012) and 60.17 µl/l air (Saad *et al.,* 2018). In another study alpha-pinene, beta-pinene and eucalyptol were required to be used at the rate of more than 10 mg/l air for 50 per cent mortality of *S.oryzae* after 48h while LC_{50} was observed to be 3.76 mg/l air for Linalool (Kim *et al.,* 2013). Sabinene hydrate exhibited 100 per cent mortality of *S.oryzae* at 12.5 and 25 mg/L air which declined to 26 per cent mortality at 6.5 mg/l air concentration after 48 h fumigation (Kim *et al.,* 2016).

In most of these studies, the combination of dose and exposure period is very high for the complete mortality of *S.oryzae* due to which it may not be used in the management of this insect.In the present investigation an attempt has been made to study the fumigant toxicity of alpha-pinene, beta-pinene, eucalyptol, linalool and sabinene against Rice Weevil, *S. oryzae* at a wide range of concentration and exposure period,so that the dose and exposure period may be optimized for the control of this insect.

MATERIALS AND METHODS

Procurement of test chemical

All the five test monoterpenes, namely, alpha-pinene, beta-pinene, eucalyptol,linalool, and sabinene were procured from Aldrich Chemistry (Sigma Aldrich Co., 3050, Spruce Street, St. Louis, MO 63103, USA, 314-771-5765).

Insect culture

Pure culture of *S. oryzae* was raised on whole wheat grain, variety PBW-502. The grain was pre-sterilised in oven at 90 ºC for 6 h to eliminate any prior infestation and its moisture was maintained to 13.5 per cent by adding required amount of water using formulae (Pixton, 1967). After mixing the water in the grain thoroughly, it was stored in air tight jar for a week for moisture equilibrium.

Quantity of water to be added = $W_1(M_2- M_1)/100-M_2$
Where,

W_1 = Initial weight of grain
M_1 = Initial moisture content
M_2 = Final moisture content

For raising the culture of test insect,100 adults of *S. oryzae* were released in Plastic Jar (1litre capacity) containing 1/2 kg of whole wheat grain (13.5 % moisture). For proper aeration, the cap of the jar was equipped with 1cm hole covered with fine wire mesh to check the escape of insects. The culture was raised at 27±1 ºC temperature and 70±5 per cent relative humidity maintained in the culture room. After 30 days, the insects released in the jar were taken out from it by sieving and their progeny was used for experimental purpose.

Fumigation bioassay

Fumigant toxicity of alpha-pinene, beta-pinene, eucalyptol, linalool, and sabinene was studied in plastic vials (115 ml capacity) at 100, 200, 300, 400 and 500 µl/l air along with un-fumigated control. Each treatment was replicated three times. Measured quantity of test chemical was soaked on blank untreated thick paper mat which was fixed in the cap of plastic vial. For comparison of fumigant toxicity, a blank mat without any chemical was used as control. Before treating the mat with test chemical, 10 adult insects of same age were released in each vial. After releasing the insects in vial, the mat was fixed in the cap by odourless synthetic glue and measured quantity of test chemical was applied on it. After application of test chemical the vial was closed with cap and it was sealed with paraffin film to make it completely airtight. Observation was recorded at 6, 12, 24, 48, 72 and 96 h after treatment without opening the vials. The mortality in insects was adjudged by observing their movements and insects showing any movement were considered as live. All the experiments were conducted at 27±1 ºC temperature and 70±5 per cent relative humidity.

Statistical analysis

The data was subjected to analysis of variance to work out significance of test, standard error of mean and critical differences between treatments. The analysed data was also depicted graphically for proper interpretation.

RESULTS AND DISCUSSION

Per cent mortality of *S. oryzae* exposed to 100, 200, 300, 400 and 500 µl/l air of alpha-pinene, beta-pinene, eucalyptol, linalool and sabinene for 6,12,24,48,72 and 96 h is presented in Table 1, 2, 3, 4, 5and 6 and Figure 1, 2, 3, 4, 5 and 6. Alpha-pinene, beta-pinene and eucalyptol caused 40 per cent mortality of *S.oryzae* at 500µl/l air after 6h fumigation while linalool and sabinene were not much effective during this period (Table 1; Figure 1). Remarkable effect of all the monoterpenes was observed after 12h exposure during which mortality increased in all the treatments. Exposure of *S. oryzae* to 500 µl/l air

Table 1: Per cent mortality of *S. oryzae* after 6h exposure

Essential oil	Concentration (µl/l air)				
	100	200	300	400	500
Alpha-pinene	0.00[a]	0.00[b]	20.00[ab]	30.00[a]	40.00[a]
Beta-pinene	0.00[a]	23.33[a]	30.00[a]	40.00[a]	40.00[a]
Eucalyptol	0.00[a]	0.00[b]	10.00[bc]	16.67[a]	40.00[a]
Linalool	0.00[a]	0.00[b]	0.00[c]	0.00[c]	0.00[b]
Sabinene	0.00[a]	0.00[b]	0.00[c]	3.33[c]	6.67[b]
SEm±	-	1.49	5.77	4.22	4.71
CD (0.05)	NS	4.70	18.19	13.29	14.85

* Means followed by the same letter are at par with each other

Table 2: Per cent mortality of *S. oryzae* after 12h exposure

Essential oils	Concentration (µl/l air)				
	100	200	300	400	500
Alpha-pinene	10.00[a]	40.00[b]	66.67[ab]	76.67[ab]	93.33[a]
Beta-pinene	3.33[a]	60.00[a]	66.67[ab]	66.67[bc]	66.67[b]
Eucalyptol	0.00[a]	73.33[a]	83.33[a]	83.33[a]	96.67[a]
Linalool	0.00[a]	3.33[c]	6.67[c]	13.33[d]	30.00[c]
Sabinene	0.00[a]	30.00[b]	50.00[b]	60.00[c]	73.33[b]
SEm±	-	5.58	6.50	4.71	3.64
CD (0.05)	NS	17.58	20.47	14.85	12.43

* Means followed by the same letter are at par with each other

Table 3: Per cent mortality of *S. oryzae* after 24h exposure

Essential oils	Concentration (µl/l air)				
	100	200	300	400	500
Alpha-pinene	16.67[a]	73.33[ab]	93.33[a]	100.00[a]	100.00[a]
Beta-pinene	3.33[a]	93.33[a]	100.00[a]	100.00[a]	100.00[a]
Eucalyptol	10.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Linalool	3.33[a]	33.33[c]	63.33[b]	83.33[b]	90.00[a]
Sabinene	0.00[a]	63.33[b]	70.00[b]	83.33[b]	90.00[a]
SEm±	-	9.43	7.15	2.98	-
CD (0.05)	NS	29.71	22.53	9.39	NS

* Means followed by the same letter are at par with each other

Table 4: Per cent mortality of *S. oryzae* after 48h exposure

Essential oil	Concentration (µl/l air)				
	100	200	300	400	500
Alpha-pinene	83.33[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Beta-pinene	3.33[bc]	96.67[a]	100.00[a]	100.00[a]	100.00[a]
Eucalyptol	86.67[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Linalool	26.67[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Sabinene	0.00[c]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
SEm±	7.89	-	-	-	-
CD (0.05)	24.86	NS	NS	NS	NS

* Means followed by the same letter are at par with each other

eucalyptol and alpha-pinene resulted in 96.67 and 93.33 per cent mortality, respectively, after 12h fumigation while sabinene and linalool caused only 73.33 and 30.00 per cent morality at this dosage. Mortality increased with dosage in all the treatments (Table 2; Figure2).

Table 5: Per cent mortality of *S. oryzae* after 72h exposure

Essential oil	Concentration (µl/l air)				
	100	200	300	400	500
Alpha-pinene	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Beta-pinene	13.33[bc]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Eucalyptol	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Linalool	40.00[b]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Sabinene	3.33[c]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
SEm±	9.19	-	-	-	-
CD (0.05)	28.96	NS	NS	NS	NS

*Means followed by the same letter are at par with each other

Table 6: Per cent mortality of *S. oryzae* after 96h exposure

Essential oil	Concentration (µl/l air)				
	100	200	300	400	500
Alpha-pinene	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Beta-pinene	40.00[b]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Eucalyptol	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Linalool	93.33[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Sabinene	20.00[c]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
SEm±	4.71	-	-	-	-
CD (0.05)	14.85	NS	NS	NS	NS

* Means followed by the same letter are at par with each other

Enhancement in the fumigant toxicity of all the monoterpenes was recorded after 24h exposure during which eucalyptol and beta-pinene killed all the insects at 200 and 300µl/l air, respectively. Such effect was noticed at 400µl/l air in case of alpha-pinene. As compared to above mentioned chemicals, slightly low toxicity was observed in case of linalool and sabinene which killed 90.00 per cent insects at 500µl/l air (Table 3; Figure 3) .

Further improvement in fumigant toxicity of all the monoterpenes was noticed after 48h exposure during which alpha-pinene, eucalyptol, linalool and sabinene killed all the insects at 200µl/l air while 96.67 per cent mortality was caused by beta-pinene at this dose. However, beta-pinene also achieved 100 per cent mortality at 300µl/l air during this period (Table 4; Figure 4). In the next observations recorded at 72h after fumigation, alpha-pinene and eucalyptol killed all the insects even at 100µl/l air while very low efficacy was noticed in case of beta-pinene linalool and sabinene at this level (Table 5; Figure 5). Significant improvement in the efficacy of linalool was recorded after 96h during which it killed 93.33 per cent insects at 100µl/l air while beta-pinene and sabinene remained ineffective at 100µl/l air even after such a long exposure (Table 6; Figure 6).

The result indicated that fumigant toxicity of mono-terpenes depends upon dose and exposure period. Similar observations have also been observed by other workers (Sriti *et al*., 2017). In the present investigation 83.33 per cent mortality of *S.oryzae* was observed at 100 µl/l air of

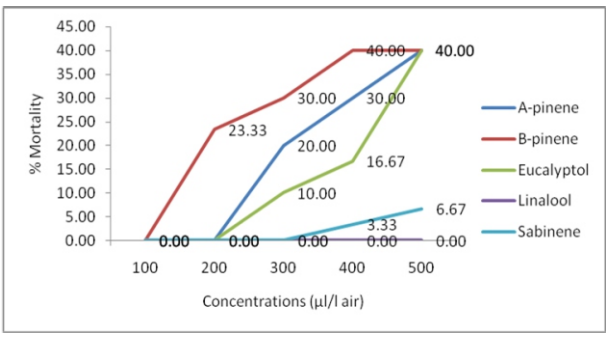

Figure 1: Efficacy of different monoterpenes after 6 h exposure

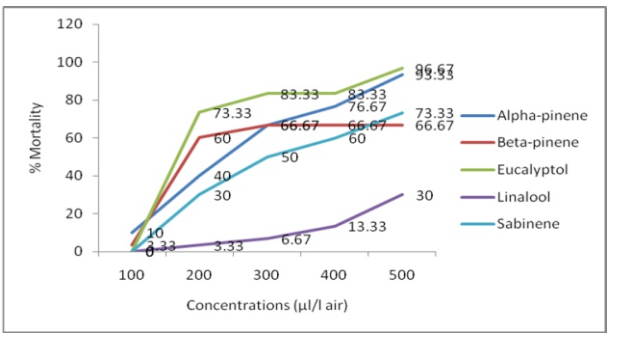

Figure 2: Efficacy of different monoterpenes after 12h exposure

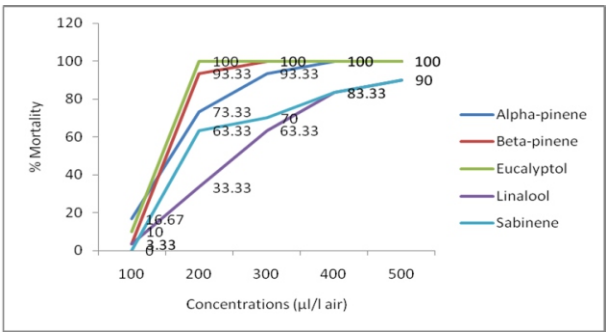

Figure 3: Efficacy of different monoterpenes after 24h exposure

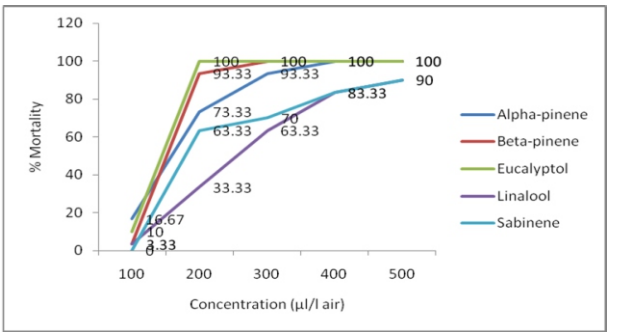

Figure 4: Efficacy of different monoterpenes after 48h exposure

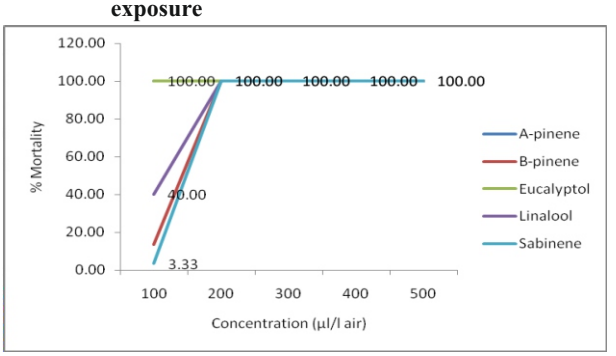

Figure 5: Efficacy of different monoterpenes after 72h exposure

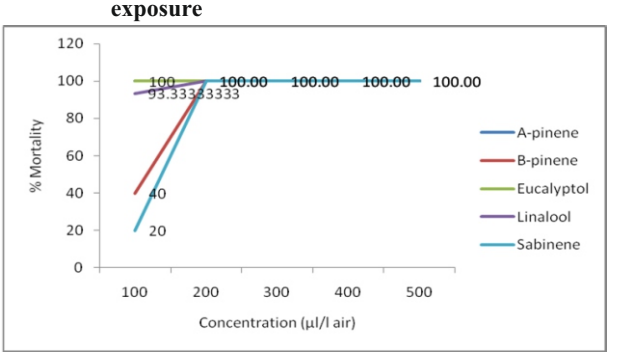

Figure 6: Efficacy of different monoterpenes after 96h exposure

alpha-pinene which increased to 100 per cent at 200 µl/l air, after 48 h of exposure. The result indicated that alpha-pinene is not much effective at lower concentration as reported by Kim *et al.* (2016) who could not get any mortality after 48 h exposure at 25 mg/l. Contrary to it Kim *et al.* (2013) have earlier observed LC_{50} value >10 mg/l of air after 48 h after fumigation. Chaubey *et al.*(2012) also reported that an exposure of *S. oryzae* at 1190 µl/l of alpha-pinene for 24 h is required for 50 per cent mortality. Saad *et al.* (2018) estimated LC_{50} value 60.17 µl/l after 24 h exposure. Similar results were also obtained for beta-pinene against *S. oryzae* (Kim *et al.,* 2013; Kim *et al.,* 2016). However, in present investigation only 3.33 per cent mortality was observed at 100 µl/l air which increased to 96.67, 100, 100 and 100 per cent at 200, 300, 400 and 500 µl/l air after 48 h exposure.

In the study conducted by Ho Lee *et al*.(2004),1,8-Cineole (eucalyptol) caused 95.3,94.1 and 99.6 per cent reduction in emergence of adults when eggs were exposed to 200µl/l air for 7 days. However, in the present investigation all the insects died within 24h when they were exposed to 200µl/l eucalyptol. In the study conducted by Kim *et al.* (2013) and Kim *et al.* (2016), the fumigant toxicity of eucalyptol was at par to alpha and beta-pinene.

As compared to previous studies (Kim *et al.,* 2013 and Kim *et al.,* 2016), marked difference was noticed in the present study with respect to fumigant toxicity of linalool against *S. oryzae*. LC_{50} value of 3.76 mg/l air was

estimated against *S. oryzae* adult after 48 h fumigation (Kim *et al.,* 2013) while 100 per cent mortality was observed after 48 h of linalool exposure at 25 mg/l (Kim *et al.,* 2016). However, in the present study linalool showed only 93.33 per cent mortality at 100µl/l after 96 h. Most interestingly, complete mortality of *S. oryzae* was seen with in 48 h when concentration of linalool was increased to 200µl/l.

The fumigant toxicity of sabinene against *S. oryzae* was not as good as reported by earlier workers. In the present study sabinene showed 100 per cent mortality at 200 µl/l after 48 h exposure while it was completely ineffective at 100 µl/l. Contrary to it Kim *et al.* (2016) observed 100 per cent mortality of *S. oryzae* adults at 25 mg/l after 48 h exposure. The difference in the efficacy may be due to quality of test material or susceptibility of insect species to it.

CONCLUSION

The present study revealed that all the test monoterpenes were toxic to adults of *S. oryzae*; however, remarkable difference was noticed in their relative efficacy against this insect. Eucalyptol which killed all the insects at 200µl/l air after 24h exposure may be adjudged as most toxic followed by beta pine and alpha pinene which exhibited such high level of mortality at 300 and 400µl/l air during the same exposure. With high mortality at 100µl/l air after 24, 48, 72 and 96h exposure, linalool may be treated as more toxic as compared to sabinene.

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