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BIOCONTROL POTENTIAL OF SELECTED PLANT ESSENTIAL OIL CONSTITUENTS AS FUMIGANTS OF INSECT PESTS ATTACKING STORED FOOD COMMODITIES.

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Abstract

Laboratory space fumigation studies were conducted to evaluate the fumigant toxicity of selected essential oil terpenoids against adult *Sitophilus oryzae* L., *Rhyzopertha dominica* F., *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* L. and *Callosobruchus chinensis* F. Five essential oil constituents, alpha humulene, caryophyllene oxide, myrcene, R-(+)-alpha pinene and R-(+)-beta pinene were each evaluated at four rates (0, 1, 5 and 10 µl/L air) in space fumigation chambers with four replicates per concentration. Results showed strong dose-, insect species- and time-dependent fumigant toxicity in which caryophyllene oxide, myrcene, α- humulene, R- (+)-α- pinene and R- (+)-β- pinene caused 18- 100, 49- 100, 55- 100, 47- 100 and 33- 100% kill of all test insects, except the most tolerant species, *T. castaneum*, at 10 µl/L air 168 h after treatment. Except *T. castaneum*, end-point LC50 values of 0.03- 8.5, 0.03- 7.0, 0.01- 4.82, 0.01-8.20 and 0.03- 6.5 µl/L air were obtained for the five terpenoids, respectively. The varied toxicities could be explained by the compound structure-insecticidal activity relationships that influence their degree of penetration into the insect cuticle and neurotoxicity. These findings provide the scientific basis for using essential oils as fumigants against storage insects and hence, potential alternative fumigants in both subsistence and commercial agriculture. Further studies are recommended to evaluate the grain fumigation potency, biosafety and broad spectrum bioactivity of these essential constituents against insect pests of stored food commodities.

Key words: *Callosobruchus chinensis*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum*, Essential oil constituent, Fumigant toxicity.

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Introduction

Poor storage and post-harvest handling practices remain major challenges facing mankind today. The food situation is made worrisome in the tropics where insect pests cause up to av. 40% pre- and post-harvest food grain losses. Grain storage and post-harvest handling plays a key role in the human food pipeline by influencing the quality and spatial-temporal availability of food grains (food supply) and also directly affects food security. Despite the phenomenal scientific breakthroughs which have resulted in increased crop productivity, insect pests greatly undermine the provision of quality food with 10-60% losses in countries where modern storage technologies are yet to be fully adopted (Shaaya et al., 1997; Ogendo et al., 2003). The magnitude of loss is dependent upon the insect species, storage duration and pest control methods among other factors. In the tropics, the gelechiid moths and coleopteran beetles are the major insect pests of stored cereal and legume grains with *Sitotroga cerealella* Olivier, *Sitophilus* spp., *Prostephanus truncatus* Horn, *Rhyzopertha dominica* F., *Tribolium castaneum* Herbst, *Acanthoscelides obtectus* Say and *Callosobruchus* spp. being the most destructive species (Ogendo et al., 2003; Gressel et al., 2004). Currently, recommended pest control measures in durable stored food products rely heavily on use of synthetic insecticides, which pose health hazards to warm-blooded animals, risk of environmental pollution, development of resistance by insects and pest resurgence, and are largely incompatible with subsistence agriculture (Shaaya et al., 1997; Prates et al., 1998; Ogendo et al., 2003; Shaaya & Kostyukovsky, 2006).

There has been a growing interest in the use of aromatic plant-based derivatives, particularly plant oils, for the protection of agricultural products due to their relative biosafety, affordability, low mammalian toxicity and eco-friendliness compared to the synthetic chemicals (Papachristos & Stamopoulos, 2002; Ketoh et al., 2005; Talukder, 2006). Available data shows that methyl iodide and allyl acetate at 3.02 mg/L and 50-150 mg/L within 24-120 h exposures achieved 100% kill of *S. zeamais* (Faruki et al., 2005; Rajendran & Muralidharan, 2005). Documented past pilot studies have shown that fairly low essential oil concentrations (50-100 g oil/M3 grain) were effective in controlling gelechiid moths and coleopteran beetles (Kostyukovsky et al., 2002; Lee et al., 2004). In their recent grain fumigation studies at 100 µl/L air, Lee et al. (2004) reported an LT50 value of 9.1 h for 1, 8-cineole against adult *S. oryzae*. Hence essential oils of botanical origin and their volatile constituents are promising alternatives to synthetic fumigants for stored-product insect pest control. Little or no local research efforts have been directed towards bioefficacy of plant essential oilconstituents. The current study evaluated the fumigant efficacy of five terpenoid essential oil constituents against adult stages of major insect pests of stored food commodities.

Materials and Methods

Test Insects

Laboratory cultures of *Sitophilus oryzae* (L.) (5-10d), *Rhyzopertha dominica* (F.) (5-10d), *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), and *Callosobruchus chinensis* (L.) (0-3d) maintained at 30±2^{o}C and 68±2% r.h. were used. Test insects were reared on soft wheat grains (*S. oryzae*, *R. dominica*), wholemeal wheat flour plus 5% brewer's yeast (19:1) (*T. castaneum*), ground wheat enriched with glycerine and yeast (*O. surinamensis*) and whole chickpea grains (*C. chinensis*).

Test Essential Oil Constituents

The essential oil constituents (standards) used in the laboratory bioactivity studies were obtained from Sigma-Aldrich Israel Ltd. Except for (-)-Caryophyllene oxide in which 90% technical grade was sourced, the GC standard was used for alpha humulene (98%), myrcene, (+)-alpha pinene and (+)-beta pinene.

Fumigant Toxicity Studies

The fumigant toxicity of five terpenoid essential oil constituents, alpha humulene, (-)-caryophyllene oxide, myrcene, (+)-alpha pinene and (+)-beta pinene, were evaluated in space fumigation according to Shaaya et al. (1991). Twenty unsexed adults of each test insect species were introduced into meshed metallic cages with a small amount of food and suspended from a hook in 3.4 L flat bottom glass space fumigation chambers. Essential oil constituents were applied separately at four dosages (0, 1, 5 and 10 µl/L air) on small pieces of Whatman No. 1 filter paper and then suspended in the chamber slightly below the cage. Magnetic stirrers were used to ensure even distribution of fumigant over a 24 h exposure time. The numbers of dead (ND) insects were recorded 24, 72, 120 and 168 h from onset of space fumigation. The number of dead (ND) and total (NT) were used to compute actual percent adult insect mortality according to Asawalam et al. (2006) and then corrected for natural mortality using Abbott's formula (Abbott, 1925).

All data on corrected percent adult mortality were first homogenized using arcsine transformations before being subjected to a multi-factorial ANOVA and treatment means separated by Tukey's studentized (HSD) test (Mead et al., 1994; Rajendran and Muralidharan, 2005; Rozman et al., 2007). Data obtained from various dose-response bioassays were further subjected to probit analysis using EPA Probit Analysis Program version 1.4 and LC_{50} values and corresponding 95% fiducial limits obtained from derived regression equations (Finney, 1971; Wheeler et al., 2007). The LC_{50} values were considered significantly different when 95% fiducial limits did not overlap.

Results

Results showed that fumigant toxicity was significantly ($P<0.0001$) influenced by essential oil constituent, concentration, time after treatment, insect species and corresponding factor interactions. At the highest concentration (10 µl/L air) and 24 h after treatment, alpha humulene, caryophyllene oxide and myrcene caused 3.0- 85.0, 6.0- 32.0 and 0- 26.0% kill of the five test insects with corresponding LC_{50} values of 0.48- 135.8, 29.6- 65.8 and 42.7- 145.0 µl/L air, respectively (Fig. 1; Table 1A). With the exception of *T. castaneum*, (+)-alpha pinene and (+)-beta pinene caused 8.0- 89.0 and 1.0- 79.0% mortality of the other four test insect species under similar conditions with LC_{50} values 5.11-70.1 and 2.94-59.4 µl/L air, respectively (Fig. 2; Table 1B). At 10 µl/L air and 24 h after treatment, inter-compound comparisons showed that *C. chinensis* and *T. castaneum* were the most susceptible (26.0- 89.0% kill) and tolerant (0- 8.0% kill) insect species, respectively. The cumulative percent mortality of all insects tested were higher 168 h after treatment with essential oil constituents compared to 24 h (Tables 1A and 1B).

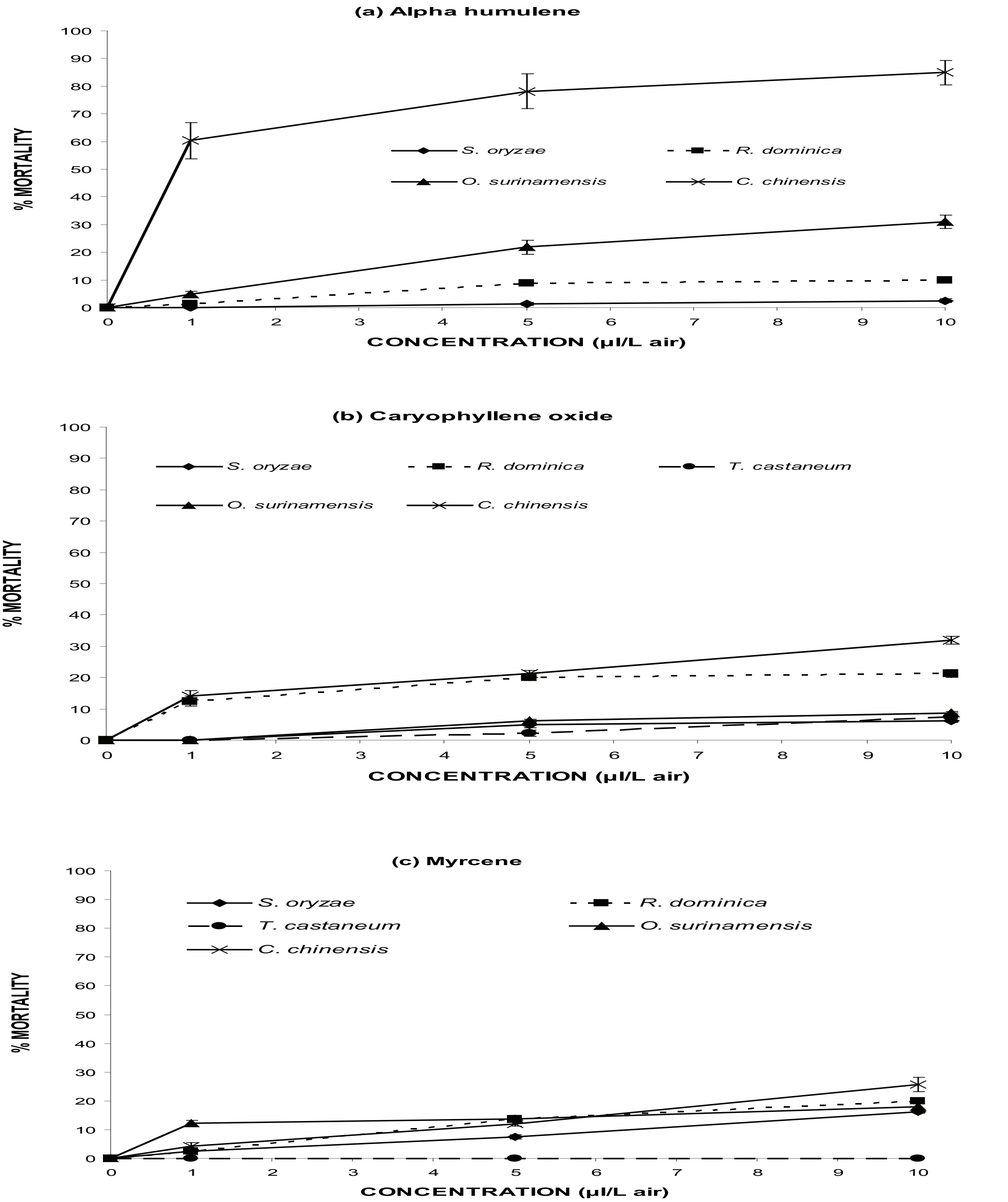

Figure 1
Percentage Adult Mortality (Mean ± SE, N= 4) of Five Stored-product Insect Pests 24h After Treatment with Ocimum gratissimum L. essential Oil constituents (a) alpha humulene, (b)caryophyllene oxide and (c) myrcene in Space fumigation Chambers over a 24-h Exposure Time.

Table 1 A

Variation of LC_{50} Values (µl/L Air) for Alpha Humulene, (-)-Caryophyllene Oxide and Myrcene With Respect to Time and Insect Species in Space Fumigation Chambers1.

Essential oil constituent/Insect species	LC_{50} values a (µl/L air) based on plant part and time (hours) after treatment							
	24		72		120		168	
	LC_{50}	95% FL	LC_{50}	95% FL	LC_{50}	95% FL	LC_{50}	95% FL
α-humulene:								
S. oryzae	135.80	(47.6,66)	6.91	(3.59,9.72)	5.42	(3.77,10.9)	3.85	(2.05,6.15)
R. dominica	106.30	(32.1,149)	27.5	(14.1,47.5)	22.2	(13.4,63.6)	9.80	(6.65,19.2)
*T. castaneum**	-	-	-	-	-	-	-	-
C. chinensis	0.48	(0.08,0.9)	0.01	(0.00,0.10)	-	-	-	-
O. surinamensis	25.7	(14.7,89.2)	7.22	(5.3, 11.1)	4.82	(3.51, 6.9)	4.20	(3.02, 6.0)
Caryophyllene oxide:								
S. oryzae	65.84	(34.2, 282)	47.10	(36.2, 56.7)	31.50	(22.4, 37.7)	28.40	(19.9, 33.4)
R. dominica	32.48	(14.9, 49.8)	0.52	(0.01, 1.42)	0.20		0.07	(0.01, 0.28)
T. castaneum	58.22	(21.9, 62.0)	40.06	(28.8, 47.7)	28.30	(0.03, 0.46)	28.30	(15.9, 32.7)
C. chinensis	29.56	(14.0, 43.7)	5.25	(3.06, 5.04)	-	(15.9, 32.7)	-	-
O. surinamensis	NS	-	NS	-	NS	-	78.0	(58.9, 100)
Myrcene:								
S. oryzae	145.01	(36.5, 102)	138.0	(33.4, 219)	22.7	(12.4, 52.9)	13.1	(8.41, 31.1)
R. dominica	61.77	(24.6, 110)	13.9	(8.04, 49.8)	0.19		0.11	(0.00, 0.61)
T. castaneum	NS	-	NS	-	58.2	(0.01, 0.96)	29.5	(16.8, 54.5)
C. chinensis	42.67	(20.3, 51.4)	7.14	(4.77, 25.6)	-		-	-
O. surinamensis	NS	-	NS	-	NS	(21.9, 97.8)	17.04	(9.27, 36.6)

NS = Response to essential oil constituent insignificant; 95% FL = 95% fiducial limits for LC_{50} values.
1Twenty unsexed adult test insects, in four replicates, were exposed to fumigant in space fumigation chambers for a 24 h.
aLC_{50} values considered significantly different when 95% fiducial limits do not overlap.

Table 1B

Variation of LC50 Values (µl/L Air) for (+)-Alpha Pinene and (+)-Beta Pinene with Respect to Time and Insect Species in Space Fumigation Chambers1.

Essential oil constituent/ Insect species	LC_{50} valuesa (µl/L air) based on plant part and time (hours) after treatment							
	24		72		120		168	
	LC_{50}	95% FL	LC_{50}	95% FL	LC_{50}	95% FL	LC_{50}	95% FL
(+)-α-pinene:								
S. oryzae	NS	-	104.07	(30.3, 142)	6.87	(4.55, 8.95)	4.41	(3.68, 5.29)
R. dominica	36.81	(25.4, 55.8)	8.55	(3.90, 20.6)	0.47	(0.02, 1.18)	0.02	(0.00, 0.11)
*T. castaneum**	-	-	-	-	-	-	-	-
C. chinensis	5.11	(3.12, 7.25)	1.69	(0.87, 2.58)	-	-	-	-
O. surinamensis	70.1	(22.4, 168)	14.4	(6.69, 65.2)	8.20	(5.7, 14.8)	8.20	(5.7, 14.8)
(+)-β-pinene:								
S. oryzae	51.95	(33.7, 65.9)	33.70	(28.3, 51.5)	10.58	(3.49, 15.6)	5.26	(2.80, 7.79)
R. dominica	12.30	(9.66, 21.4)	1.15	(0.11, 2.30)	0.12	(0.01, 0.41)	0.03	(0.00, 0.20)
*T. castaneum**	-	-	-	-	-	-	-	-
C. chinensis	2.94	(1.17, 3.06)	0.56	(0.14, 0.88)	-	-	-	-
O. surinamensis	59.4	(21.7. 178)	31.1	(14.7, 138)	8.60	(6.23, 14.1)	5.44	(4.1, 7.6)

NS = Response to essential oil constituent insignificant; 95% FL = 95% fiducial limits for LC_{50} values.
1Twenty unsexed adult test insects, in four replicates, were exposed to fumigant in space fumigation chambers for a 24 h.
aLC_{50} values considered significantly different when 95% fiducial limits do not overlap.

The results showed existence of clearly discernible dose-dependent compound variations in the fumigant toxicity against adult stages of the test insects. At 10 µl/L air and 168 h after treatment, adult *R. dominica* insects were the most susceptible (LC_{50} values: 0.02- 0.11 µl/L air) in space fumigation with all compounds except alpha humulene (LC_{50} value: 9.80 µl/L air). Against adult *S. oryzae*, alpha humulene (LC_{50} value: 3.85 µl/L air) and caryophyllene oxide (LC_{50} value: 28.40 µl/L air), were the most and least toxic compounds, respectively.

Similar result trends, although varied in magnitude, were observed for these compounds against adult *O. surinamensis.* Inter-insect species comparisons, irrespective of concentration and time after treatment, showed that *O. surimanensis* (9.0- 26.0% mortality) and *C. chinensis* (47.0-100%), were the most tolerant and susceptible insect species, respectively. On the basis of end-point LC_{50} values, (+)-beta pinene (0.03- 5.44 µl/L air) was the most efficacious followed by alpha pinene (0.02-8.20 µl/L air), alpha humulene (3.85-9.80 µl/L air), myrcene (0.11-29.5 µl/L air) and caryophyllene oxide (0.07-78.0 µl/L air), respectively.

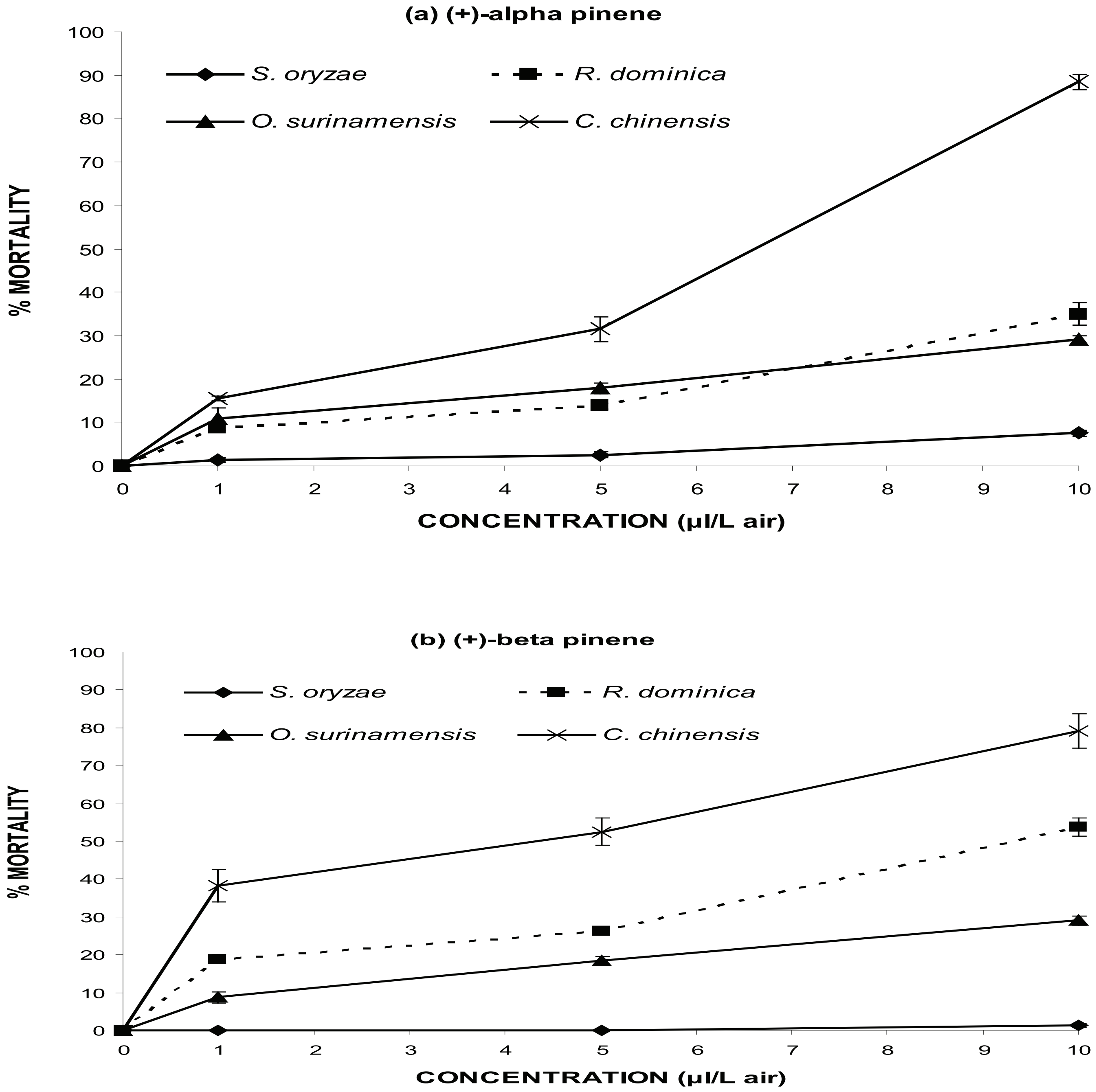

Figure 2
Percent Adult Mortality (Mean ± SE, N= 4) of Four Stored-Product Insect Pests 24 H After Treatment with Two Terpenoid Compounds, (a) (+)-Alpha Pinene and (b) (+)-Beta Pinene in Space Fumigation Chambers

Discussion

The current study has shown that the five terpenoid essential oil constituents had compound- and species-specific fumigant efficacies that were highly dependent upon the dosage and time after treatment. The fact that the five terpenoid essential oil constituents achieved 50% kill of all test insects, except *T. castaneum*, at concentration range of 0.48- 145.0 µl/L air 24 h after treatment, offers scientific stimulus for a practical non-chemical alternatives to synthetic fumigants against insect pests of stored food grains. The moderate to strong fumigant toxicities of the five terpenoid essential oil constituents could possibly be due to their differential compound structure-activity relationships and inter-insect species' responses as manifested in physiological-structural induced cellular changes resulting in poisoning of insects by blocking octopamine receptors (Ogendo et al., 2008). The observed fumigant toxicity (LC_{50} values: 0.48- 145 µl/L air within 24 h) of five terpenoid essential oil constituents, alpha humulene, caryophyllene oxide, (+)-alpha pinene, (+)-beta pinene and myrcene were inferior to that of other essential oil constituents, eugenol, (Z)-β-ocimene and 1,8-cineole. Space fumigation studies with 1,8-cineole, at 0.14 µl/L air, recorded 100 and 97.5% mortality of adult S. oryzae and *R. dominica*, respectively (Rozman et al., 2007). Similarly, Ogendo et al. (2008) reported that eugenol and (Z)-β-ocimene, at 10 µl/L air and 24 h after treatment, caused 76- 100 and 69-100% kill of all test insects, except *T. castaneum*, with corresponding LC_{50} values of 0.03- 8.21 and 0.28- 11.77 µl/L air, respectively. In earlier studies,

Prates et al. (1998) reported strong insecticidal activity of two monoterpenes, 1, 8-cineole and R-(+)-limonene, against *R. dominica* and *T. castaneum*. The LC_{50} values (21.2 µl/L air) reported in this study for *T. castaneum* fall within the range (3.5 and 466 µl/L air) observed against *T. confusum* in recent studies (Stamopoulos et al., 2007).

Conclusion

It is clear from the findings of this study that the five terpenoid essential oil constituents had moderate to strong fumigant toxicity against adult stages of five major insect pests of stored food commodities and are therefore candidate fumigants of stored durable agricultural products. However, further in-depth bioactivity (contact toxicity, reproduction inhibition, repellence, phytotoxicity, feeding deterrence and quality), grain fumigation, biosafety and socio-economic studies with a view to adopting plant essential oils and their constituents as alternatives to synthetic fumigants, methyl bromide and phosphine, for stored-product insect control protocols in tropical agriculture.

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TOXIC, ANTIFEEDANT AND REPELLENT ACTIVITY OF AQUEOUS CRUDE EXTRACTS OF *Tephrosia vogelii* HOOK ON THE LARVAL STAGES OF *Helicoverpa armigera* HÜBNER

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Abstract

Laboratory bioassays were conducted to evaluate the bioactivity of aqueous crude extracts of *Tephrosia vogelii* Hook against *Helicoverpa armigera* Hübner larvae. Fresh chickpea leaves, immersed in aqueous crude extracts of *Tephrosia vogelii* at four rates (0, 5, 10 and 20% w/v), were assayed for toxic, antifeedant and repellent effects against 2nd and 3rd instar larvae of *H. armigera* in a completely randomized design (CRD) with 3-5 replicates per treatment. Ordinary water and Dimethoate (Rogor E40) ® at 2% v/v were included as negative and positive controls, respectively. Data on corrected percent mortality, repellence and deterrence coefficient were first homogenized using angular transformations before being subjected to analysis of variance (ANOVA) and means separated by Tukey's HSD test. Results showed that the toxic, antifeedant and repellent effects of crude aqueous extracts of *T.vogelii* against *H. armigera* larvae were significantly ($P<0.0001$) influenced by intra-plant variability, concentration applied, duration (hours) and corresponding factor interactions. At the highest concentration of 20% w/v, the aqueous crude extracts obtained from the leaves (22%) and pods/flowers (av. 11%) of *T.vogelii* were weakly toxic. In the antifeedant bioassay, leaf extracts caused the highest reduction (96%) in weight of larvae followed by pods/flowers (79%) and succulent stems (2.5%), respectively. There were corresponding reductions in larval feeding as the concentration of aqueous crude extracts increased. In the repellence test, except for leaf and pod/flower extracts at 20% w/v and 1 h exposure that produced moderate percent repellence (41.67%) against the larvae, there was a dose- and exposure time-dependent attraction of *H. armigera* larvae to chickpea leaves (food) treated with aqueous extracts of *T. vogelii*. The plant offers hope as a potential cost-effective and environmentally benign antifeedant for *H. armigera* control in chickpea.

Key words: *Tephrosia vogelii*, *Helicoverpa armigera*, toxicity, antifeedant, repellence.

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