Egyptian Journal of Biological Pest Control, 15(1), 2005, 27-33

Antimicrobial Activity of Certain Essential Oils against Hindgut Symbionts of the Dry wood Termite *Kalotermes flavicollis* Fabr. and Prevalent Fungi on Termite-Infested Wood

Alfazairy, Ahlam A. M.
Department of Entomology, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt
(Received, April 11, 2005; Accepted, May 23, 2005)

ABSTRACT

When the kalotermitid *Kalotermes flavicollis* Fabr. pseudergates exposed to *Casuarina*-wood wafers treated separately with different concentrations (5, 15, and 30µ l/2g wood wafer) of the subject essential oils, their spirochaete and flagellate populations abnormally reduced in numbers and vigour. The lophomonadids *Joenia* sp. and *Mesojoenia* sp. were the most adversely affected flagellates followed by the polymastigid *Foaina* sp. and the trichomonadid *Tricercomitus* sp. The *Taxodium distichum* essential oil evidently appeared to have the greatest adverse effect on test flagellates (95-100% decline in numbers within 2 to 4 days) followed by the *Eucalyptus citriodora* (89-100%) and the *Cupressus sempervirens* oils (31-100%). While with the hindgut spirochaetes, the adverse effect seemed to be reversed where the *E.citriodora* essential oil had the greatest effect (54-100%) followed by *C.sempervirens* (46-100%) and *T.distichum* (8-100%) oils. Also, the subject essential oils were assayed for their antifungal activity, at 15, 20, 25, 30, and 50µl/1ml acetone/15ml Czapek-Dox medium against isolates of four wood rotting fungi associated with *Casuarina* timber, *Aspergillus* sp. *Penicillium* sp., *Fusarium* sp. and *Mucor* sp., by a method based on inhibition of the fungus growth on agar plates. *E. citriodora* exhibited the most potent fungicidal activity against the tested four fungi, followed by *T. distichum* oil. While the *C .sempervirens* essential oil appeared to have the least significant antifungal property.

Key Words: Termites, *Kalotermes flavicollis*, symbionts, flagellates, spirochaetes, essential oils, *Taxodium distichum*, *Eucalyptus citriodora*, *Cupressus sempervirens*, wood rotting fungi, symbionticidal activity, fungicidal activity.

INTRODUCTION

Termites are usually considered as pests of crops, trees, buildings and any wooden structures. Lower termites harbor in their guts populations of microorganisms known to be indispensable for their survival, being responsible, at least partially, for the digestion of cellulose, the main item of termite diet (Nunes and Dickinson, 1997). All lower termites contain a symbiotic flagellate community in their hindguts. Early and recent studies showed that hindgut cellulolytic protozoa are critical to the survival of lower termites on a diet of sound wood or cellulose (Breznak and Brune, 1994). These protozoa, which can together account for 1/3 the total weight of the insect (Katzin and Kirby, 1939), endocytose particles of wood or cellulose into food vacuoles and ferment the polysaccharide components to acetate, CO_2 and H_2. Acetate is taken up from the termite hindgut and oxidized to CO_2 and H_2O by termite tissues, a process that can support up to 100% of the respiratory requirement of some species (Odelson and Breznak, 1983; O'Brien and Breznak, 1984). Acetate, propionate, and other organic acids produced during microbial fermentation of carbohydrate in the hindgut are important oxidizable energy sources for termites (Odelson and Breznak, 1983), as well as carbon skeletons for biosynthesis (Blomquist *et al.*, 1979 and 1982; Guo *et al.*, 1991).

The use of naturally occurring anti-termite compounds (essential oils), extracted from locally available plants or trees, showed promise approaches to termite control (Chaboussou, 1979; Lin and Wang, 1991; Wilkins, 1992; Tienshu and Lin, 1998; Gurdip *et al.*, 2001 and 2002; Tellez *et al.*, 2001 and 2002; Zhu *et al.*, 2001; Maistrello and Henderson, 2000; Maistrello *et al.*, 2003; Peterson and Wilson, 2003). These facts draw attention to ask, should these phytochemicals have a detrimental effect on the hindgut protozoa? The present study aims to cast light on: (1) the antisymbiont activity of three essential oils extracted from leaves or seeds of medicinal timber trees (the Egyptian cypress *Cupressus sempervirens* L., the lemon-scented gum *Eucalyptus citriodora* Hook F. and the baldcypress *Taxodium distichum* L.) against the symbiotic flagellates and spirochaetes found in the hindgut paunch of the dry wood termite *Kalotermes flavicollis* Fabr.; (2) their antifungal activity against isolates of *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp. and *Mucor* sp. associated with termite-infested *Casuarina's* timber.

MATERIALS AND METHODS

I- Evaluation of the symbionticidal activity of certain essential oils

A laboratory culture of termite *Kalotermes flavicollis* Fabr (Isoptera: Kalotermitidae) was kept at 23-29°C and 71-86%R.H. in blackened glass jars on slightly moistened shavings or wafers (ca., 0.1 cm thick) of sound timber of *Casuarina* sp. (Alfazairy and Hassan, 1988). Previous trials (Alfazairy *et al.*, 2001) proved the termiticidal activity of the tested essential oils. The essential oils were extracted by water distillation of leaves of the Egyptian cypress *Cupressus sempervirens* L., the lemon-scented gum *Eucalyptus citriodora* Hook, and seeds of the baldcypress *Taxodium distichum* L. (Guenther, 1961; Anonymous, 1968; Gunther and Joseph, 1978).

Bioassay was carried out by dipping a wafer (ca., 2g and 1mm thick) of the *Casuarina* timber in a clean and sterile Petri dish (9 by 1.5cm) containing the tested concentration of the essential oil dissolved in acetone (as a carrier solvent; Yun and Burkholder, 1981; Singh *et al.*, 1989). Test concentrations of 5, 15 and 30µl/1ml solvent/2g wood wafer were tested. The wood wafers were thoroughly steeped with each test concentration by means of a sterile forceps and manual shaking to ensure a uniform exposure of wafers to the essential oils. All acetone was soaked up by the wafer, so that all oil in the acetone solution ended up in the wood wafer.

The wafers were air-dried for 30min, and after the acetone had completely evaporated, twenty pseudergates of *K. flavicollis* were introduced in each test Petri dish. A control group of wood wafers was treated with acetone only and tested after the acetone had completely evaporated too. Each test concentration was replicated six times. All Petri dishes were kept between two sheets of black paper, under the same previously mentioned laboratory conditions. Two of moribund termites were removed after 1, 2, 4, and 10 day post-treatment for the presence and count of the viable protozoa and spirochaetes in the hindgut paunch of the subject termites. Another two healthy termites which served as control were removed, and the same micro-biota in the hindgut paunch were checked and recorded. Also, the symbionticidal effect of the tested three essential oils on vigor of *K .flavicollis*-hindgut symbionts was determined by examining the movements of the symbionts by light microscopy. The hindgut paunch was removed with a dissecting needle. The paunch was placed into 50µl of 0.9% saline solution (NaCl) and agitated (Khoo and Sherman, 1979; Mankowski and Morrell, 1993). Then 5µl of the mixture was pipetted onto a haemocytometer, and the viable flagellates and spirochaetes were counted per 5µl saline solution.

The data were subjected to analysis of variance (ANOVA), and significant differences among treatment means were determined with Duncan's new multiple range test at $P = 0.05$.

II- Evaluation of the antifungal activity of the tested essential oils

The subject essential oils of *C .sempervirens*, *E. citriodora* and *T.distichum* were also tested for their fungicidal or fungistatic activity against isolates of four fungi associated with *Casuarina* timber, *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Mucor* sp.; at concentrations of 15, 20, 25, 30 and 50µl/1ml acetone/15ml Czapek-Dox (C-D) medium. Under aseptic conditions, C-D-treated Petri dishes were prepared by pipetting the tested volume of each essential oil in a clean, sterile Petri dish containing 1ml acetone and thoroughly dissolved, then a volume of 15ml C-D medium was poured into, and by means of gentle movements of the Petri dish, the test oil was thoroughly incorporated into the C-D medium. Then all dishes were left to solidify. By means of a black marker, each Petri dish was divided, from outside, into four quarters. Each quarter was inoculated by a loopful of the spore suspension of each tested fungus (1.4×10^8 spore/ml). Each test concentration of each essential oil was replicated three times. Ten C-D-Petri dishes served as control were inoculated exactly the same way as the treated ones, but without any essential oil. All Petri dishes were incubated at 28±1°C. Growth of the fungi on agar plates was examined two days post-incubation to determine whether the tested substances had fungicidal activity against the subject fungi or not (Narenda and Singh, 1985).

RESULTS AND DISCUSSION

I- The adverse effect of the tested essential oils on symbiotic spirochaetes and protozoa in the kalotermitid termite *Kalotermes flavicollis*

Spirochaetes and four species of symbiotic protozoa (Fig. 1) were identified from the hindgut paunch of pseudergates of the kalotermitid termite *K. flavicollis*. The protozoan flagellates are belonging to the orders polymastigida and hypermastigida; following the genera *Foaina, Joenia, Mesojoenia,* and *Tricercomitus* (Kudo, 1939 and 1971; Yamin, 1979; Lavette, 1980).

When the *K.flavicollis* pseudergates exposed to *Casuarina*-wood wafers treated separately with different concentrations (5, 15, and 30µl/2g wood wafer) of essential oils of *Taxodium distichum* (Td), *Eucalyptus citriodora (Ec),* and *Cupressus sempervirens (*Cs), their spirochaete and protozoan populations abnormally reduced in numbers and vigor. The adverse effect of the tested essential oils on *K. flavicollis* symbiotic microbiota seemed, in general, to be oil variety, post-treatment period, and dose dependent. The data in Table 1 provide evidence that with the highest dose of Td (30µl/2g wood wafer) and at 1, 2, and 4 days post-treatment, the hindgut spirochaetes of *K. flavicollis*-moribund pseudergates were killed; whereas with essential oils of Ec, spirochaetes were significantly reduced in numbers, and completely absent by 10 days post-treatment with Td, Ec, and Cs at high or low tested concentrations.

The exposure of *K. flavicollis* pseudergates to *Casuarina* wood wafers treated separately with the highest test concentration (30µl/2g wood wafer) of the subject essential oils resulted in the death of nearly 41, 90, 97 and 100% of their hindgut protozoa 1, 2, 4 and 10 days post-treatment, respectively. While the corresponding rates of flagellate depletion with the tested concentration 15µl, at 2, 4 and 10 days after treatment were nearly 62, 89 and 100%, in respect. Symbiotic protozoan fauna also declined in termites exposed to *Casuarina* wood-shavings treated with the essential oils of concentration 5µl 2-, 4-, and 10- day post-treatment, the decline in the flagellate numbers was nearly by 15, 75, and 100%, respectively. However, at one day post-treatment, certain hindgut symbionts (spirochaetes and flagellates) were either increased (significantly) or decreased (insignificantly) in their numbers (Table 1). The reason of this increase is unclear, although the test conditions may have enhanced reproduction of these symbionts.

Of the four symbiotic flagellates found in *K. flavicollis* pseudergates, the lophomonadids *Joenia* sp. and *Mesojoenia* sp. were the most adversely affected flagellates followed by the polymastigid *Foaina* sp. and the trichomonadid *Tricercomitus* sp. (Table 1). On the other hand, of the three essential oils that adversely affected the *K. flavicollis* pseudergates hindgut symbionts, the Td essential oil evidently appeared to have the greatest effect on their flagellates (95-100% decline in numbers within 2 to 4 days) followed by Ec (89-100%) and Cs (31-100%) essential oils. While with the hindgut spirochaetes, the adverse effect seemed to be reversed, where the Ec essential oil had the greatest

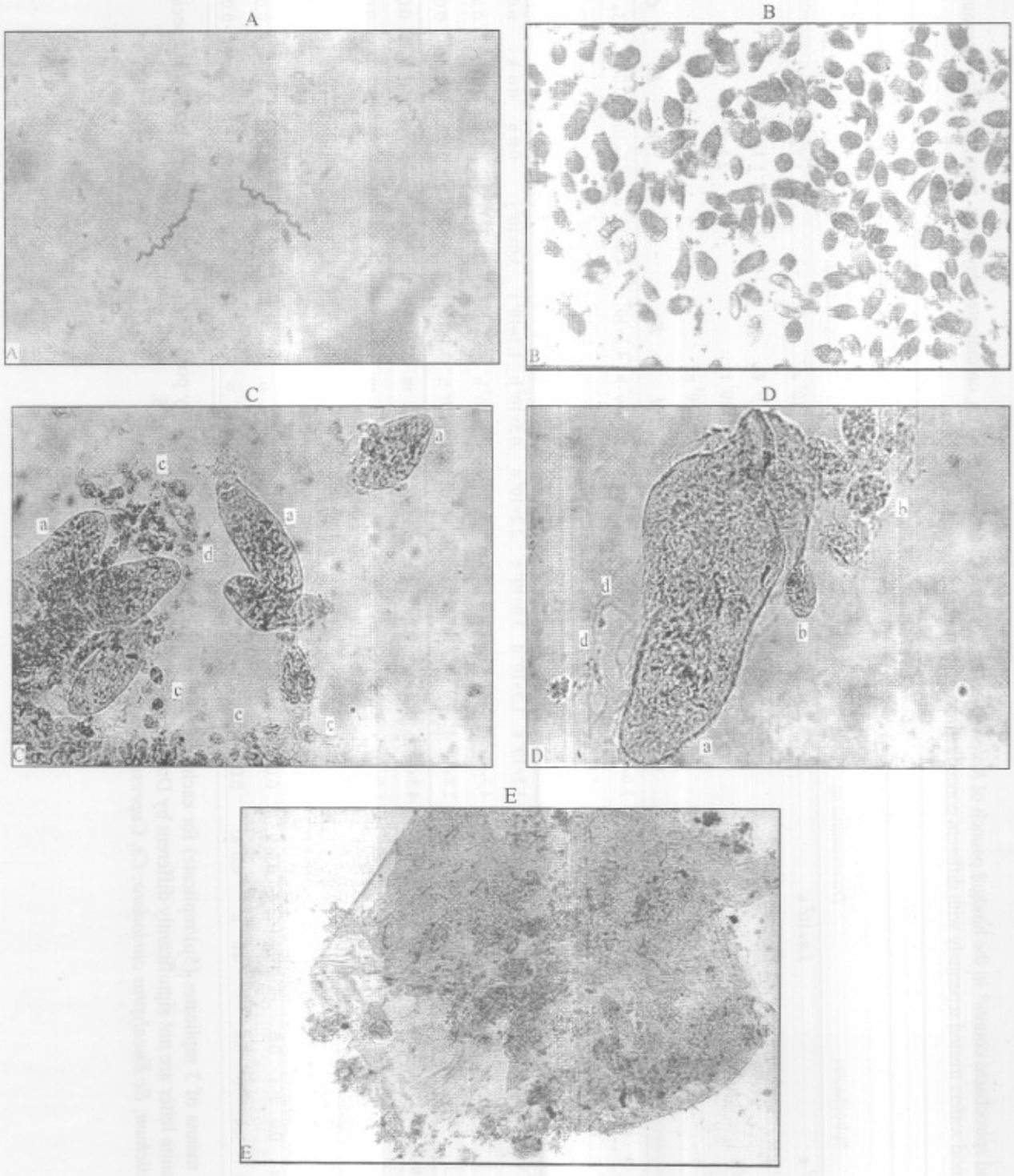

Fig. (1): Symbiotic spirochaetes and flagellates that live in the hindgut paunch of *Kalotermes flavicollis* pseudergates. A, spirochaetes, X 480; B, flagellates, X 100; C; D, the same (X 480; X 1080, respectively; a, *Joenia* sp.; b, *Mesojoenia* sp.; c, *Foaina* sp.; d, *Tricercomitus* sp.); E, the hindgut paunch of a moribund termite showing the adverse effect of the tested essential oils on numbers of its symbionts.

Table (1): Flagellate and spirochaete counts* in the hindgut paunch of *Kalotermes flavicollis*-moribund pseudergates, on indicated days post- treatment, after exposure to *Casuarina*-wood wafers treated separately with different concentrations of three essential oils**

Days post-treatment	Conc. µl/2g wood wafer	Microbiota														
					Flagellates											
		Spirochaetes			*Tricercomitus* sp.			*Foaina* sp.			*Mesojoenia* sp.			*Joenia* sp.		
1	O (control)	1.4×10^3 a			2.8×10^2 a			7.0×10^2 a			8.5×10^2 a			1.7×10^3 a		
2		2.6×10^3 e			1.8×10^2 d			1.4×10^3c			3.1×10^3 g			1.1×10^3 g		
4		3.8×10^3 l			2.0×10^2 g			2.5×10^3 h			1.4×10^3 l			2.3×10^3 i		
10		4.1×10^3 s			1.8×10^3 j			2.3×10^3 l			1.6×10^3 n			2.0×10^3 k		
		Td	***Ec***	***Cs***	***Td***	***Ec***	***Cs***	***Td***	***Ec***	***Cs***	***Td***	***Ec***	***Cs***	***Td***	***Ec***	***Cs***
1	5	5.5×10^3 b	1.8×10^3 a	1.8×10^3 a	1.8×10^2 a	5.5×10^2 a	3.0×10^3 b	5.5×10^2 a	6.5×10^2 a	2.4×10^3 b	1.5×10^2 b	4.3×10^2 c	4.8×10^2 d	8.5×10^2 b	0.0 c	7.5×10^2 d
	15	3.4×10^3 c	1.5×10^3 a	1.6×10^3 a	0.0 c	3.5×10^2 a	1.5×10^3 a	1.8×10^2 a	4.0×10^2 a	5.5×10^2 a	1.3×10^2 b	2.8×10^2 e	3.5×10^2 df	3.5×10^2 e	0.0 c	4.0×10^2 d
	30	0.0 d	1.3×10^3 a	1.3×10^2 a	0.0 c	1.3×10^2 a	4.0×10^2 a	0.5×10^2 a	3.0×10^2 a	3.5×10^2 a	1.0×10^2 b	1.8×10^2 e	2.8×10^2 f	0.0 f	0.0 c	3.0×10^2 d
2	5	2.4×10^3 e	1.2×10^3 f	1.4×10^3 g	1.0×10^2 d	2.0×10^2 d	1.5×10^3 e	1.5×10^2 d	3.0×10^2 e	2.2×10^3 c	0.5×10^2 h	1.3×10^2 i	3.0×10^2 j	0.0 h	0.0 h	0.0 h
	15	3.5×10^2 h	8.5×10^2 fk	3.5×10^2 i	0.0 f	1.0×10^2 d	4.3×10^2 d	0.8×10^2 d	1.5×10^2 e	1.2×10^3 c	0.3×10^2 h	0.8×10^2 i	1.3×10^2 j	0.0 h	0.0 h	0.0 h
	30	0.0 j	1.3×10^2 k	1.0×10^2 i	0.0 f	0.3×10^2 d	2.8×10^2 d	0.0 f	0.8×10^2 e	1.3×10^2 g	0.3×10^2 h	0.5×10^2 i	0.0 k	0.0 h	0.0 h	0.0 h
4	5	1.6×10^3 m	1.5×10^2 n	1.0×10^2 o	0.8×10^2 g	1.3×10^2 g	4.3×10^2 h	0.3×10^2 I	1.0×10^2 i	8.5×10^2 h	0.0 m	0.0 m	0.0 m	0.0 j	0.0 j	0.0 j
	15	1.0×10^2 p	1.0×10^2 n	1.3×10^2 o	0.0 i	0.5×10^2 g	1.5×10^2 g	0.5×10^2 I	0.5×10^2 i	4.3×10^2 hk	0.0 m	0.0 m	0.0 m	0.0 j	0.0 j	0.0 j
	30	0.0 q	0.8×10^2 n	0.3×10^2 o	0.0 i	0.0 i	0.8×10^2 g	0.0 j	0.0 j	1.0×10^2 k	0.0 m	0.0 m	0.0 m	0.0 j	0.0 j	0.0 j
10	5	0.0 t	0.0 t	0.0 t	0.0 k	0.0 k	0.0 k	0.0 m	0.0 m	0.0 m	0.0 o	0.0 o	0.0 o	0.0 l	0.0 l	0.0 l
	15	0.0 t	0.0 t	0.0 t	0.0 k	0.0 k	0.0 k	0.0 m	0.0 m	0.0 m	0.0 o	0.0 o	0.0 o	0.0 l	0.0 l	0.0 l
	30	0.0 t	0.0 t	0.0 t	0.0 k	0.0 k	0.0 k	0.0 m	0.0 m	0.0 m	0.0 o	0.0 o	0.0 o	0.0 l	0.0 l	0.0 l

* Values represent means of 3 replicates (5µl/replicate) for each test termite. Means at each indicated day post-treatment for a given microbiota species followed by the same letter are not significantly different by Duncan's new multiple range test at P = 0.05.

** *Td*, *Taxodium distichum*; *Ec Eucalyptus citriodora*; *Cs*, *Cupressus sempervirens*.

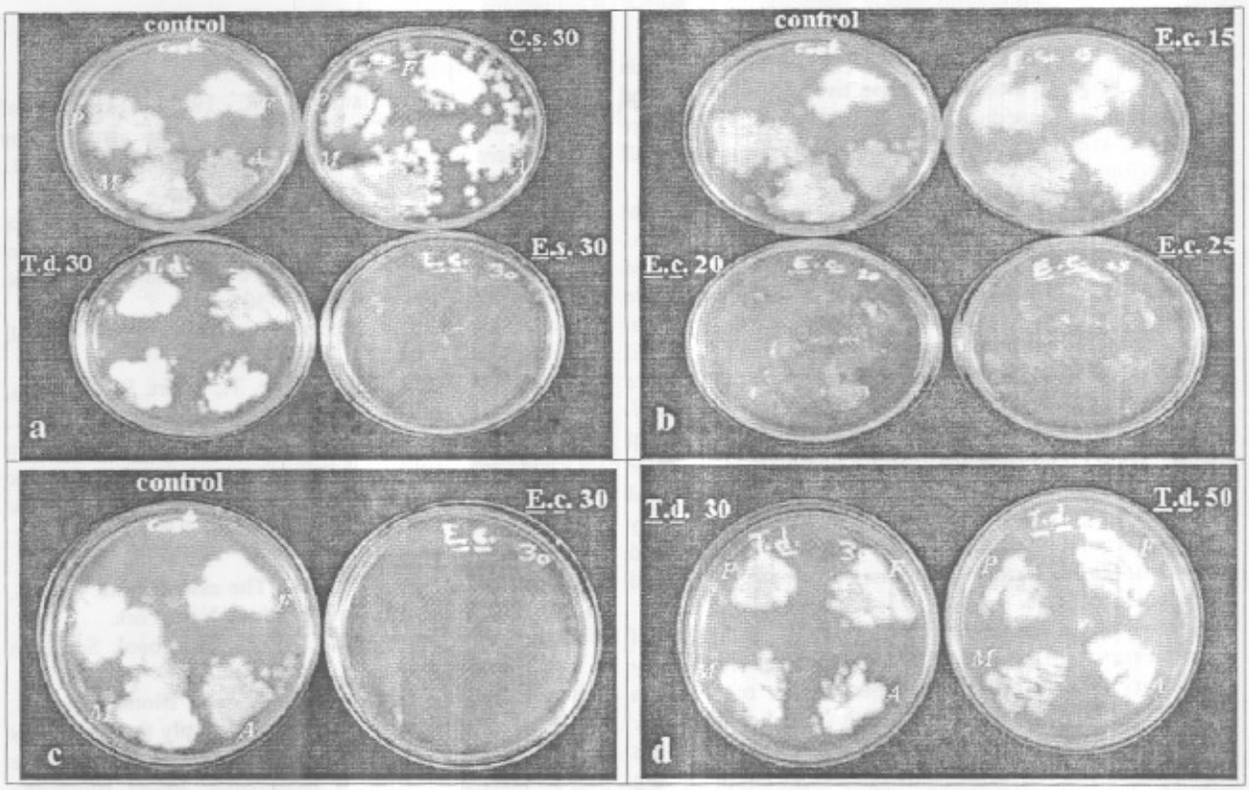

Fig. (2): Degree of growth inhibition of the four wood rotting fungi, *Aspergillus* sp. (*A*), *Penicillium* sp. (*P*), *Fusarium* sp. (*F*) and *Mucor* sp. (*M*), 2 days post-incubation in agar medium (Czapek-Dox) treated separately with different concentrations (15, 20, 25, 30 and 50µl/1ml acetone/15ml medium) of *Cupressus sempervirens* (C.s.), *Eucalyptus citriodora* (E.c.) and *Taxodium distichum* (T.d.) essential oils.

effect (54-100%) followed by Cs (46-100%) and Td (8-100%) oils.

By examining the movements of both the spirochaetes and the flagellates (by light microscopy) in the hindgut paunch of *K .flavicollis*-moribund pseudergates, resulted from exposure to *Casuarina*-wood shavings treated with different concentrations of the tested essential oils, the movement or vigor of these symbionts was significantly less than that of untreated termites. The present depletion and sharp drop (1-4 day post-treatment) of the *K. flavicollis* symbiotic spirochaetes and flagellates by the subject essential oils may reflect their direct toxic effect on numbers and vigor of these symbionts. The progressive depletion and death of the symbiont population living inside the termite hindgut caused host starvation (Alfazairy *et al*., 2001) which resulted in the death of all treated termites in 11 days. Alfazairy *et al*. (2001) mentioned that the present essential oils seemed to act as feeding deterrents to *K.flavicollis* termites; therefore, it appears that the essential oils of *Td*, *Ec* and *Cs* may act both directly and indirectly to reduce vigor and numbers of symbiotic spirochaetes and flagellates of *K.flavicollis* pseudergates. Such an adverse, direct or indirect, effect of essential oils on termite symbionts has previously been reported by Carter (1977), Carter and Mauldin (1983), and Carter *et al.* (1983), Maistrello and Henderson (2000), and Maistrello *et al*., (2003).

II- Fungicidal activity of the tested essential oils

Penicillium sp., *Aspergillus* sp., *Fusarium* sp. and *Mucor* sp. are prevalent fungi on termite-infested *Casuarina* timber in Alexandria, Egypt, and cause considerable damage to these timbers (woodrotting fungi). The present essential oils of *Td*, *Ec* and *Cs* were laboratory bioassayed for their termiticidal activity against the present kalotermitid pest, *K.flavicollis* by Alfazairy *et al.* (2001). In the present study, the same essential oils were tested for their antifungal activity at concentrations of 15, 20, 25, 30 and 50µl/15ml C-D medium against the forenamed fungi by a method based on inhibition of the fungus growth on agar plates (Narenda and Singh, 1985).

Degree of inhibition of the four fungi after 48hrs of exposure to the tested essential oils that thoroughly incorporated into the C-D medium is illustrated in Figure 2. The total or partial absence of the fungal growth after seeding on the treated C-D medium was the criterion for inhibition. Growth of the tested fungi was slightly to completely inhibited by the subject essential oils in a dose-dependent manner, where the antifungal properties of all essential oils increased with an increase in concentration (Fig. 2b and d).

The *Td* and *Ec* oils were inhibitory to both mycelial growth and sporulation of the four fungi. The essential oil of *Cs* had a slight inhibitory effect (Fig. 2a). The

minimum inhibitory concentration of the *Td* and *Ec* oils against the tested fungi was, respectively, 50 (Fig. 2d) and 15µl/15 ml C-D medium (Fig. 2b). At 30µl/15 ml culture medium, the *Ec* oil exhibited complete inhibition for all fungi (Fig. 2a; c).

The results showed that *Ec* oil exhibited the highest fungicidal activity against *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp. and *Mucor* sp., followed by *Td* oil, and higher concentrations gave better inhibitory effects. While the *Cs* oil appeared to have the least significant antifungal property. The antifungal properties of the *Ec* essential oil against strains of *Aspergillus* spp and *Fusarium* spp. were previously reported by Lokesha *et al.*, (1986) Paran *et al.* (1996), Assawah (2002), Cimanga *et al.* (2002), Pradeep *et al.* (2003). and Salgado *et al* (2003). Also, a slight or no inhibitory effect of the *Cs* oil on some isolates of *Aspergillus* spp and *Penicillium* spp. was observed by Siddiqui *et al.* (1996). But Okasha *et al.* (1997) mentioned that *Aspergillus niger* and *Penicillium* sp. exhibited resistance to *Td* oil.

Taking into consideration the termiticidal activity of the present three essential oils that has been reported by Alfazairy *et al.* (2001), and observations of Ruyooka (1979) on the addition of fungi to the wood considerably reduced its resistance to termites, the present findings would emphasize the potentials of these oils in protecting the *Casuarina* timbers against *K .flavicollis* termites by their fungicidal and termiticidal activities, as well as the present symbionticidal activity. Also, the tested essential oils provide evidence for their termiticidal, symbionticidal and fungicidal activities.

REFERENCES

Alfazairy, A.A. and Hassan, F.A. 1988. Infection of termites by *Spodoptera littoralis* nuclear polyhedrosis virus. Insect Sci. Appl., 9: 37-39.

Alfazairy, A.A.; Salem, A.G. and Hassan, F.A. 2001. Termiticidal activity of essential oils extracted from some medicinal timber trees against the dry wood termite *Kalotermes flavicollis* Fabr. (Isoptera: Kalotermitidae). J. Adv. Agric. Res., 6(3): 695-709.

Anonymous. 1968. Determination of volatile oil in drugs. Published by the Pharmaceutical Press, London, W.C.I.

Assawah, S. 2002. Effect of some plant extracts and essential oils on *Fusarium* wilt of broad bean. African J. of Mycol. & Biotechnol., 10(3): 75-86.

Blomquist, G.J.; Howard, R.W. and McDaniel, C.A. 1979. Biosynthesis of cuticular hydrocarbons of the termite *Zootermopsis angusticollis* (Hagen). Incorporation of propionate into dimethylalkanes. Insect Biochem., 9: 371-374.

Blomquist, G.J.; Dwyer, L.A.; Chu, A.J.; Ryan, R.O. and de Renobales, M. 1982. Biosynthesis of linoleic acid in a termite, cockroach and cricket. Insect Biochem., 12: 349-353.

Breznak, J. A. and Brune, A. 1994. Role of microorganisms in the digestion of lignocellulose by termites. Ann. Rev. Entomol., 39: 453-487.

Carter, F.L. 1977. Responses of subterranean termites to wood extractives. Rev. Appl. Entomol., 65(6): 3405.

Carter, F.L. and Mauldin, J.K. 1983. Responses of *Reticulitermes flavipes* to extracts from nine antitermitic hardwoods. Rev. Appl. Entomol., 71, 1889.

Carter, F.L.; Jones, S.C.; Mauldin, J.K. and Camargo, C.R.R. 1983. Responses of *Coptotermes formosanus* Shiraki to extracts from five Brazilian hardwoods. Rev. Appl. Entomol., 71, 3730.

Chaboussou, F. 1979. Recent progress in termite control. Rev. Appl. Entomol., 67(6): 2388.

Cimanga, K.; Apers, S.; Bruyne, T.; Miert, S.; Hermans, N.; Totte, J.; Pieters, L.; Vlietinck, A.J.; Kambu, K.; Tona, L. and Bruyne, T. 2002.Chemical composition and antifungal activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J. Essential Oil Research, 14(5): 382-387.

Guenther, E. 1961. Oil of mint. The essential oil. Vol. III, D. Van Nostrand Company, Inc., New York.

Gunther, Z. and Joseph, S. 1978. "Handbook Series in Chromatography" CRC Press, Inc., New York.

Guo, L.; Quilici, D.R.; Chase, J. and Blomquist, G.L. 1991. Gut tract microorganisms supply the precursors for methylbranched hydrocarbon biosynthesis in the termite, *Zootermopsis nevadensis*. Insect Biochem., 21: 327-333.

Gurdip, S.; Singh, O.P.; Rao, P.; Singh, P.K.; Pandey, K.P. and Singh, G. 2001. Studies on essential oils, Part 29: Insecticidal activity of some volatile oils and monoterpenoids against white termite (*Odontotermes obesus* Rhamb.). Sugar Cane International, November, 18-20.

Gurdip, S.; Singh, O.P.; Prasad, Y.R.; Lampasona, M.P.; Catalan, C. and Singh, G. 2002. Studies on essential oils, Part 33: Chemical and insecticidal investigations on leaf oil of *Coleus amboinicus* Lour. Flavour and Fragrance Journal, 17(6): 440-442.

Katzin, L.I. and Kirby, H. Jr. 1939. The relative weights of termites and their protozoa. J. Parasitol., 25: 444-445.

Khoo, B.K. and Sherman, M. 1979. Toxicity of chlorpyrifos to normal and defaunated Formosan subterranean termites. J. Econ. Entomol., 72(2): 298-304.

Kudo, R.R. 1939. Protozoology. Bailliere, Tindall and Cox, London, pp. 260-287.

Kudo, R.R. 1971. Protozoology. Charles C. Thomas Publisher, Springfield, Illinois, U.S.A., pp. 457-487, Fifth edition.

Lavette, A. 1980. The ultrastructure of the zooflagellates of the genera *Foaina* and *Pyrsonympha* inhabiting termites and the cellular coaptations. Annales de Sciences Naturelles, Zoologie et Biologie Animale, 2(1): 1-17.

Lin, T.S. and Wang, C.L. 1991. The anti-termite properties of extracts from *Melia azedarach* Linn. Rev. Agric. Entomol., 79(10): 9807.

Lokesha, S.; Vasantha, K. and Shetty, H.S. 1986. Effect of plant extracts on growth and sporulation of *Aspergillus flavus*. Plant Disease Research, 1(1-2): 79-81.

Maistrello, L. and Henderson, G. 2000. Vetiver grass: useful tools against formosan subterranean termites can be found in nature. Vetiver Newsletter, 22: 16-17.

Maistrello, L.; Henderson, G. and Laine, R.A. 2003. Comparative effects of vetiver oil, nootkatone and disodium octaborate tetrahydrate on *Coptotermes formosanus* and its symbiotic fauna. Pest Management Science, 59(1): 58-68.

Mankowski, M.E. and Morrell, J.J. 1993. Resistance of damp wood termites to preservative-treated wood. Forest Prod. J., 43(9): 58-60.

Narenda, S. and Singh, R.S. 1985. Inhibition of *Fusarium udum* (pigeon pea wilt pathogen) by ether distillate of margosa cake amended soil. Indian J. of Mycology and Plant Pathology, 13(3): 329-330.

Nunes, L. and Dickinson, D.J. 1997. The effect of boric acid on the protozoan numbers of the subterranean termite, *Reticulitermes lucifugus*. Rev. Agric. Entomol., 85(8): 7378.

O'Brien, R.W. and Breznak, J.A. 1984. Enzymes of acetate and glucose metabolism in termites. Insect Biochem., 14: 639-643.

Odelson, D.A. and Breznak, J.A. 1983. Volatile fatty acid production by the hind-gut microbiota of xylophagous termites. Appl. Environ. Microbiol., 45: 1602-1613.

Okasha, M.M. and Hussein, E.H.; Hassan, F.A. 1997. Microbial susceptibility and biodegradability to *Taxodium distichum* seed oil. Zagazig J. Pharm. Sci., 6(1): 41-47.

Paran, B.; Sharma, R.K.; Singh, R.S.; Ghosh, A.C. and Baruah, P. 1996. Fungicidal activity of some naturally occurring essential oils against *Fusarium moniliforme*. J. Essential Oil Research, 8(4): 411-412.

Peterson, C.J. and Wilson, E.J. 2003. Catnip essential oil as a barrier to subterranean termites (Isoptera: Rhinotermitidae) in the laboratory. J. Econ. Entomol., 96(4): 1275-1282.

Pradeep, A.G.; Lokesh, S. and Rai, V.R. 2003. Efficacy of some essential oils on seed mycoflora and seedling quality of some crop species saved by farmers. Advances in Plant Science, 16(1): 53-58.

Ruyooka, D.B.A. 1979. Association of *Nasutitermes exitiosus* (Hill) (Termitidae) and wood rotting fungi in *Eucalyptus regnans* F. Muell. and *Eucalyptus grandis* W. Hill ex Maiden: choice-feeding, laboratory study. Zeitschrift für Angewandte Entomologie, 87(4): 377-388.

Salgado, A.P.; Cardoso, M.G.; Souza, P.E.; Souza, J.A.; Abreu, C.M. and Pinto, J.E. 2003. Fungitoxic activity evaluation of essential leaf oils of *Eucalyptus* on *Fusarium oxysporum*, *Botrytis cinerea* and *Bipolaris sorokiniana*. Ciencia e Agrotecnologia, 27(2): 249-254.

Siddiqui, R.R.; Ahmad, H.; Shakoor, C.S.; Ehteshamuddin, A.F.M. and Shireen, S. 1996. Antimicrobial activity of essential oils. Part II. Pakistan J. of Scientific and Industrial Research, 39(1-4): 43-47.

Singh, D.; Siddiqui, M.S. and Sharma, S. 1989. Reproduction retardant and fumigant properties in essential oils against rice weevil (Coleoptera: Curculionidae) in stored wheat. J. Econ. Entomol., 82(3): 727-733.

Tellez, M.; Estell, R.; Fredrickson, E.; Powell, J.; Wedge, D.; Schrader, K. and Kobaisy, M. 2001. Extracts of *Flourensia cernua* (L.): volatile constituents and antifungal, antialgal, and antitermite bioactivities. J. Chemical Ecology, 27(11): 2263-2273.

Tellez, M.R.; Khan, I.A.; Kobaisy, M.; Schrader, K.K.; Dayan, F.E. and Osbrink, W. 2002. Composition of the essential oil of *Lepidium meyenii* (Walp.). Phytochemistry, 61(2): 149-155.

Tienshu, L. and Lin, T.S. 1998. Effects of essential oil from the leaves of seven *Eucalyptuses* on the control of termite. Forest Product Industries, 17: 751-760.

Wilkins, C.K. 1992. Naturally occurring anti-termite compounds. Material und Organismen, 27(1): 47-65.

Yamin, M.A. 1979. Flagellata with lower termites. Sociobiology, 4(1): 17.

Yun, T.Q. and Burkholder, W.E. 1981. Protection of stored wheat from the granary weevil by vegetable oils. J. Econ. Entomol., 74(5): 502-505.

Zhu, B.C.R.; Henderson, G.; Chen, F.; Fei, H.X. and Laine, R.A. 2001. Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. J. Chemical Ecology, 27(8): 1617-1625.