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INVESTIGATIONS ON THE POSSIBILITY OF NON-INSECTICIDAL CONTROL OF TERMITES

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CONTESTS

ABSTRACT

Effective control of termites is usually achieved by long persistent synthetic pesticides like aldrin, chlordane or heptachlor. The possibility of replacing these chlorinated hydrocarbons by some non-insecticidal methods of control were investigated in this study.

To test the effect of various substances, methods were developed for maintaining subterranean termites under laboratory conditions. The termite, ***Odontotermes guptai*** Roonwal and Bose was successfully maintained in a **B.O. D.** incubator at 25 $\pm$ 1^{o}C in glass-jars for about 6 months.

Eucalyptus seedlings upto about 1 year old are highly susceptible to attack by termites which feed on the roots. Investigations showed that the roots contained some substance which was attractive to termites. Extracts of the roots prepared in acetone and alcohol were found active as attractants in bioassay. Chemical characterization of the eucalypt root extract showed the active substance to be a phenolic acid. Further identification of the compound was not attempted. The attractancy of eucalypt root extract was not sufficiently strong to warrant testing under field conditions. Root extract of rubber seedlings, on the other hand was not attractive,

1. INTRODUCTION

Termites cause serious damage to agricultural crops,, forest trees, stored timber and other wood products. In most cases satisfatory control of termites can be achieved with long persistent synthetic pesticides, provided the right chemical is used at the appropriate time.

Among forest plantations, ***Eucalyptus*** spp. are attacked by termites during early stages of establishment, often causing considerable mortality. Field studies in Kerala (Nair and Varma 1981) have shown that usually eucalypt seedlings below one* year are attacked by termites. The mortality may be as high as 80%. The point of attack in general, is the living root system. Methods of control using cyclodiene insecticides have been standardised (Nair and Varma, 1981).

Search for pest control agents of natural origin has assumed greater importance in recent years due to the increasing cost of synthetic chemical pesticides and pollution problems. In addition, there are problems of outbreak of secondary pests and toxic effect on natural enemies of pests. In view of these problems several attempts have been made to control termites using alternative methods of control. These include use of attractant wood blocks together with a slow-acting termiticide, use of repellents or termite resistant materials and application of juvenile hormone analogues which can cause disturbance to the physiological or behavioural processes in insects. Known termite attractants include several constiruenrs of fungus-infected wood and lignin breakdown products (Smithe *et. a/.* 1967; Ritter *et. a/.* 1973; Moore 1974; Becker 1976). A sesquiterpenoid, isolated from fungus-infected wood was found to be attractive to the termite, Reticulitermes sp. Similarly extracts of the wood, Eucalyptus regnans was attractive to Nasuititermes spp. Controi of termites using a decayed wood block (which acted as an attractant) impregnated with a slow-acting insecticide to produce a toxic bait has been suggested (Esenther and Gray 1968; Esenther and Beal 1974, 1979). This method of bait blocks has been found useful in reducing the incidence of termites in buildings in residential areas in U. S. A. and Canada.

The possibility of using extracts of resistant wood species to control termites attacking stored timber has been suggested (Rudman 1965; Carter 1976; French *et al.* 1979). Extracts of the heartwood of Australian white cypress Pine *(Callitris columellaris* F. Muell) has been shown to confer resistance against termite attack and decay (Rudman 1965; French *et al.* 1979). Carter (1976) found that extractives of many temperate and tropical wood species decayed by wood-rotting fungi contained termiticidal material. Da costa *et al.* (1958) reported that 2-methyl anthraquinone (tectoquinone), which is present in the heartwood of teak prevent termite attack. In spite of these and other reports of termite repellents of plant origin, research to-date has not provided any reliable method for termite control using repellanls. The destruction of insect colonies using JH analogues has been demonstrated in ants (Cupp and O'Neal 1973: Troisi and Riddiford 1974; Edwards 1975) as well as in honey bee (Hrdy 1973; Zdarek *et al.* 1976). In termites, the effect of JH and its analogues on caste differentiation, with particular reference to soldier formation, is well established (Luscher 1969; Hrdy and Kreck1972; Wanyonyi 1974; Springhetti 1974; Varma 1977). It is suggested that termites are capable of response to and avoidance of contact with toxic substances and so compounds like JH, which will not deter termites from feeding would be advantageous (Howard and Haverty, 1979).

In the present study an attempt has been made to evaluate the attractancy of young eucalypt root to termites. Since extract of eucalypt root was found to be attractive to termites in the bioassay, studies were also made to characterise the active component present in it. Extracts of some of the commonly available plant materials were also tested for attractant or repellent effect. Experiments were also conducted to study the survival of termites on substrates treated with JH analogues (hydroprene and methoprene). For most of these experiments, laboratory cultures of termites were essential and hence methods to keep cultures of subterranean termites for a few months were standardised.

2. REARING SUBTERRANEAN TERMITES IN THE LABORATORY

Maintenance of laboratory cultures of' termites is a prerequisite for carrying out biological assay. Lower termites' (Families ***Mastotermitidae. Kalotermitidae, Hodotermitidae*** and ***Rhinotermitidae)*** can be reared comparatively easily and therefore most of the experimental work on termites has been carried out with them. On the contrary, 'Higher termites' (Family ***Termitidae)*** are difficult to rear under laboratory conditions because of their complex social organization. Successful breeding of some higher termites viz. ***Odontotermes obesus*** (Ausat *et a/.* 1962),. ***Microcerotermes beesoni*** and ***M. cameroni*** (Sen-Sarma *et a/.* 1975) has, however, been reported. A colony of ***0. obesus,*** developed from a pair of reproductives was maintained successfully over a period of two years by Ausat *et a/.* (1962). Becker (1969) has also mentioned successful breeding of some species of ***Microcerotermes*** in the laboratory. Since species of ***Odontotermes*** cause most of the damage to eucalypt seedlings in India, attempts were made to maintain laboratory cultures of the same for use in the bioassay of plant extracts.

The following three methods were tried to develop and maintain laboratory cultures of termites, in this study.

(a) Establishment of a colony from dealated reproductive pairs

Dealated reproductives of ***Odontotermes*** sp. were collected from different localities as and when swarming occurred. Thsy were released in a clean tray and those found to move in tandem were taken out and placed in pairs, in cylindrical plastic jars (10cm X 8cm), provided with a layer of moist soil at the bottom. The plastic jars were closed with a perforated lid. No food material was given since the reproductives thrive on reserve fat during the initial stages of establishment of the colony. In most cases the reproductives initiated a copularium and settled within a day. Although a number of attempts were made (Table), no colonies were established. in two instances, a few eggs were laid, but they did not hatch. All the reproductives died within about two weeks of confinement It appears most deaths occurred due to some fungus attack of unknown origin. In one of the preliminary trials in 1977, one small colony was established from a Pair of reproductives. After 48 days, the whole colony was transferred to a bigger container, which resulted in the death of the colony. As noted earlier, Ausat *et a/.* (1962) were able to establish a colony of ***Odontoterms obesus*** from a pair of reproductives which survived well over two years. The reasons for the failure of reproductive pairs to establish colonies in the present trials are not clear, The cultures were maintained at room temperature; it is possible that lower temperatures may prove beneficial.

Table 1. Survival period of alate pairs of ***Odontotermes*** sp. at room temperature

SI. No.	Date of collection	Place of collection	No of repiicates	No of days survived	Remarks
1	25—4—79	Ollukkara (Trichur)	10*	2—4	
2	22—4—79	Peechi	6	5—11	
3	'10-5-79,	Peechi	10	8—16	In two cases, eggs were laid, but did not hatch
4	26—5—79	Ollukkara (Trichur)	8	3—4	
5	30-5-79	Peechi	12	4	
6	6—7—79	Potta	15*	4-8	

* In 3 containers each, two pairs of reproductives were introduced, instead of one pair.

(b) **Maintenance of culture by transferring a portion from natural colony**

In another attempt to maintain a laboratory culture of subterranean termites, a portion of the termite mound ***(Odontotermes*** sp.) without queen was collected from field and placed in a cement tank (70cmX45cmX45cm) containing soil. The termite material introduced consisted of thousands of workers and soldiers along with fungus combs. Two openings each were provided on either side of the cement tank for ventilation which were covered with wire-gauze. Pieces of rubber wood were offered as food and water sprinkled at weekly intervals to provida moisture. The top of the cement tank was covered with a closely fitting glass plate.

In this culture, it was possible to observe the activities of the termites without disturbing the culture, by looking through the glass top. Within two days after the culture was set up, termites began their building activities. The edges of the top glass cover and also the two holes covered with wire-guaze, were found sealed off with soil particles. Construction of tunnels on the sides of the cement tank was also noticed.

After repeated trials it was found that it was not possible to develop a viable colony of Odontotermessp. by this method, The maximum period such a colony survived was 37 days (4—8—79 to 10—9—79). Apparently, absence of the queen and inability of the termites to bring about the required microclimatic conditions were responsible for the low survival period of the culture. It is reported that cultures of ***0. obesus*** consisting of all castes including the queen, collected from fields, survived over 10 months in the laboratory (Ausat *et al.* 1962).

(c) **Maintenance of small groups of subterranean termites in glass jars.**

In another attempt to culture termites in the laboratory, small groups of field-collected termites were kept in glass jars and maintained in a B.O.D incubator at 25 ± 1^{o}C. Underground nests of ***Odontotermes guptai*** located at Peechi campus were opened up, termites consisting of workers, soldiers and larvae together with nest material were collected in a tray and brought to the laboratory. The culture was set-up as follows. The glass jar (18 cm X 8cm) was filled with soil upto 4 cm height. Soil from the inner nest wall was used Two small pieces of rubber wood were partly burried into the soil for food. The fungus combs together with termites and nest debris were added and the container was closed with a perforated lid. To each jar was added abouf 5—6 grams of termites, in addition to the young larvae which usually adhere to the fungus combs. At the time of setting up the culture, the soil in the glass jar was watered slightly.

The laboratory cultures survived for varying periods (Table II) The maximum period a colony survived was 162 days.

Table II. Survival period of jar-cultures of ***O. guptai*** kept at 25 ± 1°C in the laboratory.

Date of setting up culture	No of replicates	Survival period (days)
17-6-80	5	1-17
23-7-80	5	50-69
17-9-80	5	100-158
4-4-81	12	90-162
11-5-81	10	100–138

In the active colonies the workers rearrange the mound material to a complicated system of galleries (Fig.1).

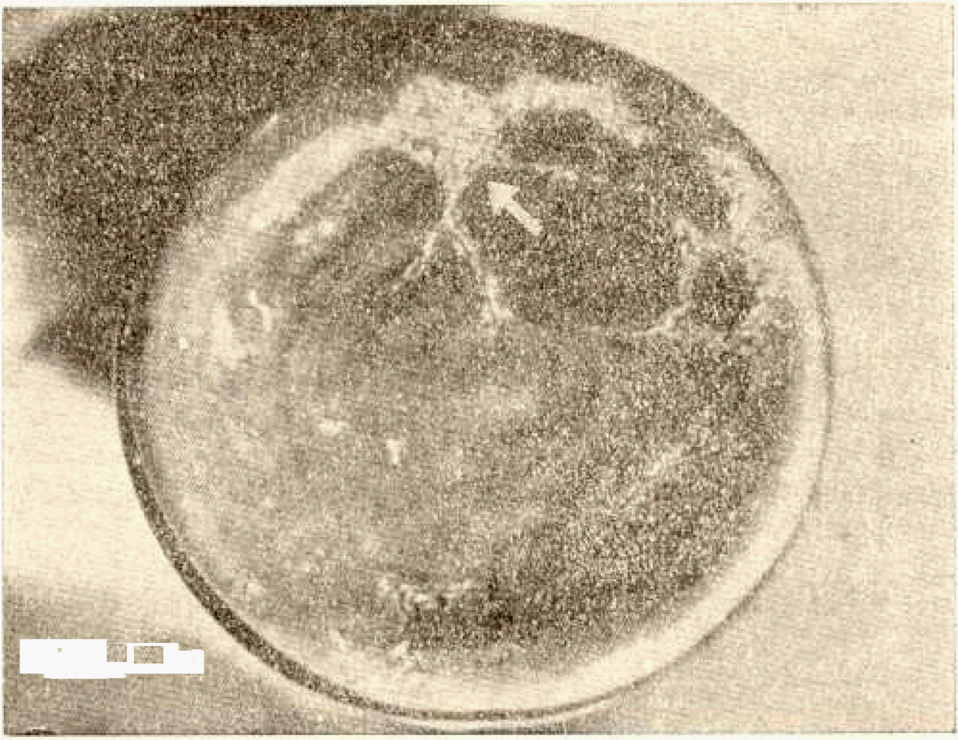

Fig. 1. Termite culture of ***O. guptai*** maintained in a glass jar at 25 ± 1°C -top view.

The workers closed even the small openings in and around the lid covering the jars. The wood pieces given as food material was also found to be cemented together with soil particles. This method of rearing was successful in keeping termites at least for 4-5 months.

The caste oomposition of 2 g samples from active jar cultures is shown in Table 111.

Table III. Caste composition in a **2** g sample from jar-cultures of *0. guptai.*

Culture No.	Workers	Soldiers	Larvae	Total
1	624	145	103	872
2	660	152	93	905
3	630	128	82	840
4	602	137	68	807
5	698	162	52	812

About 74 per cent of the termites consisted of workers, 17 per cent soldiers and 9 per cent larvae (average of 5 samples). It appears that the successful establishment of laboratory cultures in the present study is due to the use of an appropriate number and caste composition of termites at the time of setting up the culture. That tetmites will survive better in groupse than as isolated individuals has also been shown by other workers (Grasse 1946; Sen-Sarma 1964; Agarwal 1979). The longer survival period of termites in groups has been attributed to tactile stimulation, trophallaxis, clustering and regulation of factors like temperature, relative humidity etc. (Grasse and Chauvin 1944; Sen-Sarma and Kloft 1965; Pence 1956) The use of a lower temperature (25±1°c) in the present experiment may also have contributed to the survival of termite culture. All previous attempts at rearing were made at room temperature which was generally above 30".

Thus the present study shows that groups of *0. guptai* can be maintained successfully at least for a few months for use in bioassay. *Odontotermes guptai* is known to attack roots and bark of young eucalypt seedlings (Nair and Varma 1981) and hence this species is appropriate for the present investigation.

3. THE RESPONSE OF TERMITES TO EUCALYPTUS ROOT AND OTHER PLANT MATERIALS

Among forest species eucalypt seedling is one of the most susceptible to termites. Since it is known that some chemicals are repellent to termites and others attractive, it is logical to expect that the eucalypt root may contain some chemicals attractive to termites The present study was undertaken to test the attractancy of eucalypt root and other setected plant materials. One of the plant materials tested was root of rubber seedling ***(Hevea brasiliensis),*** which is not generally attacked by termites, Extracts of neem leaf, adathoda leaf and heartwood of teak were included because they have been reported to be repellent or toxic to many insects including termites (Chopra *et a/.* 1956; Ketkar 1976; Carter 1976). Leaf extract of ***Eupatorium*** odoraturn ***(Chromolaena odorata*** of King and Robinson) was also included as it is resistant to many insects. The termite used was ***Odontotermes guptai.***

Materials and Methods

Plant extracts: Details of plant materials used for the present study are given in Table IV. All root materials were freshly collected, cut into

Table **IV.** Plant-parts tested for attractant effect against ***O.guptai.***

Sr. Nn.	Family	Botanical name	Partused
1	Acanthaceae	***Adathoda Vasica*** Nees	Leaves
2	Meliaceae	***Azadirachta indica*** Linn.	Leaves
3	Compositae	***Eupotorium odoratum***	Leaves
4	Myrtaceae	***Eucalyptus tereticornis*** Smith	Root of seedlings 6 – 8 m. old and 12 – 14 m. old.
5	Myrtaceae	***Eucalyptus grandis*** Hill & Maiden	Root of seedlings 6-43 m. old.
6	Euphorbiceae	***Hevea brasiliensls*** (HBK) Arg.	Root of seedlings 6—8 m. old.
7	Verbenaceae	***Tectona grandis*** Linn.	Heartwood

small pieces and extracted in acetone using soxhlet extractor. The extracts were collected, filtered and solvent removed under low pressure. The extract was finally dried at room temperature and used for bioassay. All the leaf materials were dried at room temperature, powdered and extracted in acetone- Only acetone extracts were used since preliminary bioassay results showed that termite attractant substances could be extracted with acetone. As described in section 4, bioassay of successive extracts of root material in solvents of increasing polarity showed that termite attractant substances were present only in acetone and ethanol extracts.

Bioassay technique

The response of termites to different plant extracts was tested in single-choice and / or multiple-choice tests by recording the number of termites moving towards or away from the test material under standard conditions. The apparatus used consisted of a glass Petri-dish (15 cm diameter) to which 4 glass tubes (14 cm long) were connected at equal distance on opposite directions (Fig. 2). The open end of the tubes was closed with cork. Workers of ***Odontotermes guptai,*** from laboratory cultures

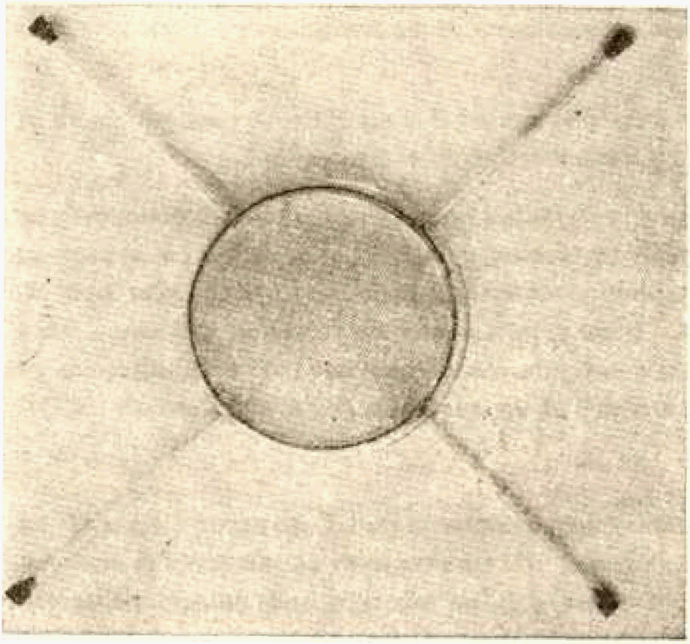

Fig. 2 Choice-test apparatus

maintained at 25 ± 1°C in a B.O.D. incubator as described earlier were used for the bioassay.

Single-choice-bioassay : The material to be tested was presented in a Whatman No. 1 filter paper strip (10 cm X 0.5 cm) placed in one of the arms of the test unit. One gram of the extract to be tested was diluted in 1 ml of acetone and spread uniformly on the filter-paper strip using a pipette. In the single-choice tests, the plant material was provided in one arm of the apparatus and a control strip, which was treated with 1 ml of the solvent alone was placed in the opposite arm. The other two arms were closed with wax. A filter-paper was placed at the bottom of the dish to facilitate easy movement of the test termites. Thirty worker termites of ***O. guptai*** were introduced into the centre of the dish and the test unit kept in the B.O.D, incubator at 25 ± 1°C. The number of termites seen in each arm of the tube at the end of 30 min was recorded. The observation time was fixed as 30 min because during preliminary tests, maximum termite response was obtained in this period. When introduced into the dish, termites hurriedly moved in different directions.

Most of them travelled in a circle. Within 5 min, most of them settled and showed investigating movements. During the half an hour observation period, some termites were always found in the central area. Each test was replicated 10 times.

A test extract was judged attractant if a significantly greater number of termites were drawn to the test extract compared to control.

Multi-choice-bioassay : In multi-choice-bioassay four different extracts were offered simultaneously by placing treated filter-paper strips in each of the four tubes and the number of termites responding to each after half an hour interval determined. Other details of the experimental procedure were the same as described for the single-choice-bioassay. The significance of the results was tested by analysis of variance (Snodecor and Cochran 1967).

Results

Single-choice-bioassay with acetone treated filter-paper strip and an untreated filter-paper strip showed that termites were not attracted to acetone Tests with extract treated paper strip on one tube and an acetone treated control strip on the opposite tube showed that termites were attracted to

Table V. Response of ***O. guptai*** to various plant extracts in single-choice bioassay.

Plant extract	No. of termites responding out of 30* rounded off to one decimal point			
	Extract treated Paper strip		Acetone treated Paper strip	
	Mean	SE	Mean	SE
E. ***tereticornis*** (6-8 months old root)	12.0**	± 1.0	2.0	± 0.5
E. tereticornis (12–14 months old root)	4.0**	± 1.0	0.7	± 0.3
E grandis (6-8 months old root)	9.0**	± 1.0	0.5	± 0.4
Rubber (6-8 months old root)	0.6	± 0.3	0.7	± 0.3
Adathoda vasica (leaf)	1.0	± 0.4	2.0	± 0.5
Eupatorium odoratum (leaf)	1.0	± 0.4	2.0	± 0.4
Neem (leaf)	1.0	± 0.4	2.0	± 0.4
Teak (sawdust)	1.0	± 0.5	1.0	± 0.4

* Mean of 10 replicates   ** Significant at 5% level.

eucalypt (6-8 months old) root extract ($P < 0.01$) (Table V). Among other root materials tested, roots of 12—14 months-old ***E. tereticornis*** were also attractive to termites. So also the root extract of ***E. grandis*** (6-8 months-old) was attractive to termites. However, the root extract (6—8 months old) of rubber was neither attractive nor repellent to termites. When the test termites were given a choice of root extract of rubber and root extract of E. ***tereticornis,*** they were drawn towards the eucalypt root extract treated filter-paper. Response to other extracts viz. ***Adathoda*** leaf, ***Eupatorium*** leaf, neem leaf and teak sawdust was not significant. When termites were allowed to choose among extracts of eucalypt root, ***Adathoda*** leaf, neem leaf and teak sawdust in a multi-choice-bioassay, more termites were drawn to eucalypt root (Table VI). This confirmed the attractiveness of eucalypt root over others.

Table VI. Response of ***0. guptai*** to various extracts in a multi-choice bioassay.

Extract	No. of termites responding out of 30. Mean of 10 replicates, rounded off to one decimal point	
	Mean	SE
E. tereticornis (6-8 months old)	10.0	± 0.7
Adathoda (leaf)	1.0	± 0.4
Neem (leaf)	1.0	± 0.4
Teak (sawdust)	0.8	± 0-3

Discussion

The present study shows that termites are attracted to eucalypt root extracts. The intensity of attraction, however, could not be assessed with accuracy. In the bioassay, only 5-18 termites out of 30 were drawn to the eucalypt root extract in the various experiments. This indicates that the eucalypt root extract is not a strong attractant. The response of individual species of termites to various chemicals can vary. Under field conditions, out of 11 species of ***Odontotermes*** spp. collected from the experimental area only 3 species including ***O. guptai*** were found to attack roots of young eucalypt seedlings (Nair and Varma 1981).

The reaction of test termite will also depend on the type of bioassay technique employed. The present bioassay technique, like many other bioassay techniques described in the literature, may not provide a natural situation for the termites and hence can affect the behavioural patterns too.

In a multi-choice-bioassay using extracts of ***Adathoda*** leaf, neem leaf and teak sawdust along with eucalypt root extract, it was found that more termites were drawn towards eucalypt root extract. It is clear that acetone extracts of other plant parts tested do not contain substance(s) attractive to termites. Presence of repellents, if any, could not be detected because only acetone extracts were tested in the present study.

The resistance of teak heartwood to termites is attributed to the presence of various anthroquinone derivatives present In it (Rudman and Gay 1961 ; Gupta and Sen-Sarma (1978) have also reported that some of the anthroquinone derivatives like crysophenol are repellent to termites. Another natural product possessing pesticidal or insect-repellent properties is the seed and leaf extracts of neem (Ketkar 1976). Warthen (1979) has shown that some of the triterpenoids isolated from neem to be a possible deterrent or growth inhibitory substance to many insects including termites. Parthenin. the major constituent derived from ***Parthenium* hysterophorus** Linn. is also reported to have insecticidal property against termites and cockroaches (Tilak 1977). However, none of these are employed at a practical level to control termites.

4. STUDIES ON THE NATURE OF TERMITE ATTRACTANT IN EUCALYPTUS ROOT

Studies reported in the previous section indicated that eucalypt root extract is attractive to termites. To elucidate the nature of the attractant substance(s) present in the eucalypt root, chemical isolation studies were undertaken.

Materials and Methods

Extractions : Using a soxhlet-extractor, 25 grams of the root material, ***E. rereticornis*** (6—8 months old), were extracted successively with Petroleum-ether (40-60) benzene, acetone and ethyl alcohol (95.5%). All the extracts were collected, filtered and the solvent removed under low pressure to a residual volume of 25 ml and stored in a vacuum desicator.

Chemical characterisation : Multiple-choice- bioassay of the various extracts showed that only acetone and alcohol extracts were attractive to termites (Tables VII & VIII). Hence these two extracts were fractionated by column and the in layer chromatography (TLC) to identify the active compound. For column chromatography silica gel G (60-120 mesh) was used and the column (15 mm X 75 mm) was packed to a height of 50 mm. The following 3 solvent systems were used — 3:2 methyl alcohol : acetone ; 3 : 1 : 1 methyl alcohol : acetone : ammonia (sp. gr. 0.91) and 9 :1 methanol : ammonia (sp. gr. 0.91). The separated fractions were collected and bioassayed for termite attractancy. Fractions showing attractancy in the bioassay were pooled and characterised on TLC. TLC plates were prepared with Silica gel G at 0.25 mm thickness and the sample developed in a 8 : 1 : 1 combination of methanol, water and ammonia. Using preparative thin layer plates (0.5 mm to 1.0 mm thickness) the detectable compound in the chtomatogram was eluted with methyl alcohol and tested for termite attractancy. Paper chromatography was also done to compare the results obtained in TLC.

Results

Multiple-choice-bioassay with eucalypt-root-extract in different solvents of both ***E. rereticornis*** and ***E grandis*** (6—8 months old) showed that only acetone and alcohol extracts elicited a positive response from workers of ***O. guptai*** (Tables VII and VIII). Both Petroleum-ether and benzene extracts of eucalypt root were not attractive. Since extracts in petroleum-ether and benzene were not attractive to termites, the presence of any liapid compound

Table VII. Response of ***O. guptai*** to root of ***E. tereticornis*** (6—8 months-old) extracted in different solvents.

Solvent	No. of termites responding out of 30*	
	Mean	SE
Petroleum-ether	0.4	± 0·3
Benzene	0-4	± 0·2
Acetone	11.0	± 1·2
Alcohol	5.0	± 1·8

Table VIII. Response of ***O. guptai*** to root of ***E. grandis*** (6—8 months-old) extracted in different solvents.

Solvent	No. of termites responding out of 30*	
	Mean	SE
Petroleum- ether	0.4	± 0.3
Benzene	0.7	± 0.3
Acetone	9.3	± 1.0
Alcohol	4.8	± 0.9

* Mean and standard error of 10 replicates, rounded off to one decimal place.

was ruled out. The presence of amino acids, amines and alkaloids, carboxylic acids, phenols, simple carbohydrates and cyanogenic glycosides were ruled out by appropriate chemical tests. When the termite attractant fraction separated from the column was chromatographed on thin layer plates, only one compound was detectable. This compound was detected as a blue spot (Fig. 3) both in TLC and paper on spraying with Folin–ciocalteu reagent and exposure to ammonia fumes with an Rf value of 87 on TLC and 91.5 on paper in 8 : 1 : 1 methanol : water : ammonia combination. It is a phenolic acid.

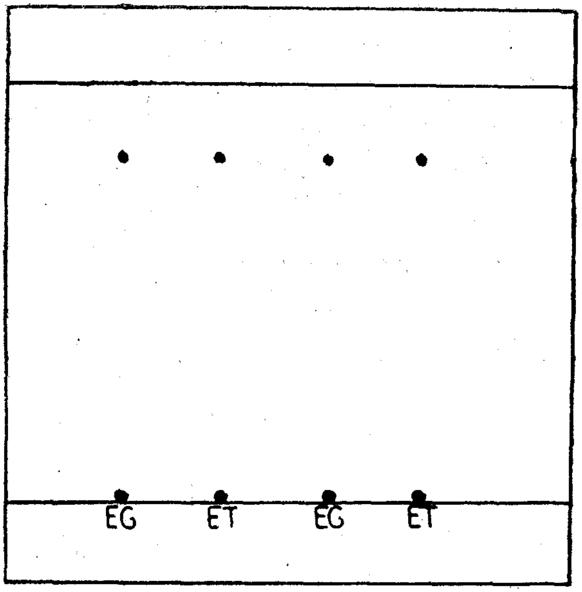

Fig 3. TLC of eucalypt root extract on silica gel **G** plate,.developad in 8 : 1 : 1 methanol : water : ammonia. Blue spots detected by spraying Folin-ciocalteu reagent and fuming with ammonia Rf-value-87

EG — ***E. grandis***

ET — ***E. tereticornis***

Discussion

The present investigations suggest that the substance responsible for termite attractancy in eucalypt root belongs to the phenolic acid group. Phenolic acids constitute a large assemblage of naturally occuring plant chemicals. The exact chemical nature of the present compound remains unknown. Becker (1964) found that vanillic, P-hydroxy-benzoic P-coumaric and protacatechnic acids were attractive to 5 different species of termites. All these compounds belong to the phenolic acid group and are known to occur in fungus-infected wood. The attraction of termites to wcod infected by the fungus ***G/oephy//um trabeum*** (Pers ex Tr) Murr. (Formerly ***Lenzites trabea.*** Perf ex. Fr., to subterranean termites is also well established (Esenther *eta/.,* 1961; Beeker. 1975). The attractant factor in the decayed wood was found to be (Z, Z. E) — 3-6, 8-dodecatrien-1-01, the production of which depends on the particular fungus culture and type of substrates used (Matsumura et el., 1972, 1976; Amburgey and Smythe 1977), Among other compounds found to be attractive to termites are cinnamyl acetate, which occurs naturally in some essential oils (Watnabe and Casida 1963) and tricycloekasantalal and dihydroagrofuran, both from sandal wood oil (Ritter *et a/,* 1973).

5. EFFECT OF JUVENILE HORMONE ANALOGUES ON TERMITES

One of the newest approaches to insect control is the use of juvenile hormone analogues (JHA) which can alter the normal physiological processes in insects. These are highly selective and do not havemany of the undesirable properties of synthetic pesticides. Since these compounds are analogues of growth regulatory chemicals naturally occurring in insects, they may be either toxic or may induce some abnormality which impairs the survival of the insect. JH analogues are also known to influence the differentiation of castes in termites (Noirot 1969; Lusoher 1972, 1976; Hrdy 1973). In the present study the objective was to investigate the effect of these chemicals and to assess the potentiality for use as control agents. The effect of JH analogue was studied by offering JH treated filter paper as feeding substrate. By feeding on the treated filterpaper, the chemical can get into the body of the insect via contact, ingestion or by trophallaxis.

Materials and Methods

Methopiene and Hydroprene were obtained through the courtesy of Dr. G. B. Stall, Zoecon Corporation, Palo Alto, California. The following concentrations of both JH analogues were used per filter paper — 1.5 μg, 1.0 μg and 0.5 μg. The required quantity of JH was dissolved in 1 ml of acetone and applied to Whatman No. 1 Filter paper (9 cm diameter). After the solvent was removed by evaporation, the filter papers were moistened with distilled water and kept in Petri-dishes (9 cm diam) The control filter paper was treated with acetone. There were two replicates of each treatment

Termites for the study were taken from laboratory cultures ***O. guptai.*** 30 active workers (age not known) from the jar-cultures were introduced into the Petri-dish. Both experimental and control group were kept in a B.O.D incubator at 25 $\pm$ 1°C.

Daily observations were taken on mortality and morphological changes, if any, in both treated and control groups. The maximum period workers of

O.guptai survived on filter paper was **15** days and hence observattons were made for a period of 2 weeks.

Results and discussion

In the petri-dish bioassay, concentrations of **1.5** and 1.00 μg per filter paper of both hydroprene and methoprene caused total mortality (Table IX). All the termites in the experimental group were found dead within 2 to **3** days. In the control group about 65 to **75%** of the termites wera active at the end of first week. However, towards the end of the second week all the termites were found dead in the control group. No morphological changes were observed in any of the group fed on treated filter paper.

Table IX. Mortality of workers of ***O.guptai*** fed on JH-treated filter papers.

Treatment	No. of insects dead out of 60 and % mortality at the end of 1 week			
	0	0.5 μg	1.0 μg	1.5 μg
Hydroprene	**14 (233)**	**26 (43.3)**	**60 (100)**	**60 (100)**
Methoprene	**20 (33**8)	**44 (73.3)**	**60** (1**00)**	**60** (1**00)**

It has been reported that methoprene and hydroprene, the two commercially available JH analogues can induce dosage dependent superflous soldier differentiation in subterranean termites (Howard and Howerty 1979; French at *a/.* 1977). The present study indicates that both these compounds are Similar in its biological activity i.e., both caused large-scale mortality at higher concentrations, The production of pre-soldiers was not observed and this to a great extent may depend on the physiological condition of the test termites. Recently Okot-Kotber (1980) has shown that in ***Macrotermes michaelseni,*** only termites of a certain age (0—6 days) will differentiate into soldiers under the influence of JH analougues.

Because of the short survival period of the test termites under our experimental conditions even in untreated controls, the present results are only of indicative value. It is, however, evident that methoprene and hydroprene are toxic to termites at higher concentrations.

6. GENERAL DISCUSSION AND CONCLUSIONS

The enormous loss caused by termites to forestry and agricultural crops needs no emphasis. Till 1946, the toxicants which were used against termites included Paris green, other arsenical compounds, paradichlorobenzene, fuel oil emulsions, coal-tar creosote and also some plant-products like extracts of tobacco leaves, neem leaves, etc. (Roonwal 1979). The use of these toxicants diminished with the advent of chlorinated hydrocarbons, which were quite effective against termites although there are residue proclems. A number of alternative control methods have been' tried in recent years to replace chlorinated hydrocarbons, but none of these methods have been found useful at a practical level so far.

Although several ant species exert some controlling effect on termites, there is only limited scope for biological control of termites using ants. The use of Fungal or bacterial pathogens is also not promising.

On the basis of results obtained in the laboratory experiments Ritter *et a/.* (1977) suggested that semiochemicals (food-attractants, trail-pheromones, etc.) in combination with termiticides offer prospects in selective control of termites. The present studies showed that the root of eucalypt seedlings contained some attractive substance to termites. Chemical studies showed that this substance was a phenolic acid. Since the biological activity of this termite attractant was low, its effectiveness under field conditions was not tested. Bioassay with rubber root extract, leaf extracts of Adathoda, neem and *Eupatorium* and teak sawdust, however, did not show attractant effect on test termites.

As a part of the present investigations, studies were also made to find out a suitable baiting material for assessing some of the attractant substances, if found. Among wood blocks (30 cm X 1.5 cm X 1.00 cm) of Bombax ceiba, ***Hevea brasiliensis*** and ***Terminalia tomentosa*** tested by partly burrying them in soil around a termite mound for 6 months, rubber wood was found to be the most susceptible. However, field tests with bait blocks, i.e. baits treated with attractants and insecticides, were not carried out since eucalypt root extract was found to be only a feeble attractant.

Juvenile hormone analogues have been found effective against social insects, because these chemicals can interfere with their normal growth processes, affect caste differentiation and also can cause adult mortality. in the present study, it was found that the two JH analogues tested viz. hydroprene and methoprene caused high mortality in laboratory tests. These compounds were, however, not tested under field conditions. They do not appear very promising under field conditions as they are known to degrade fast (Hrdy *et a/.* 1979).

The present study reveals that eucalypt root extract is only a feeble attractant and hence may not find use at a practical level in the control of termites. Sands (1977) has indicated that control of termites using biological means has only limited scope although some regulatory effect can be obtained through predators like birds, lizards and ants, mainly during termite swarms. Thus it appears that the use of soil insecticides will have to be continued for effective control of termites.

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