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Temperature Related Variations in Cuticular Hydrocarbons of *Odontotermes obesus* (Rambur) (Termitidae: Isoptera) Affect Gallery Formation and Susceptibility to Imidacloprid

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SA conceived the project and wrote the article. MSS conducted experiments in the laboratory and analyzed the data. UAF carried out experiments in the field.

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A B S T R A C T

Cuticular hydrocarbons (CHCs) of insects play an important role as communication signal and prevent environmental stresses. CHCs vary with environmental conditions. In the present studies, we have determined effect of temperature on CHCs of termites, *Odontotermes obesus* (Rambur) (Termitidae: Isoptera) and subsequent effect on gallery formation and susceptibility to the pesticide, imidacloprid. The termites' workers were collected at different times of the year, 2013-2014, from three different sites in Punjab, Pakistan and CHCs were quantified. Variations in contents of CHCs was also studied over a range of temperatures for different time points. Extracted CHCs were used to measure gallery formation. The termites' workers with low and high CHCs contents were exposed to imidacloprid and percentage mortality in each termite type was noted. Results showed that CHCs of *O. obesus* peaked at hotter months of the year (May through August) at three places which showed non-significant difference among them. CHCs increased with an increase in temperature and highest CHCs contents were observed at 35ºC. The soils treated with high contents of CHCs were probed longer by termites' workers and mortality by imidacloprid was low in termites' workers with high CHCs contents. The control strategy based on these results is suggested in discussion.

INTRODUCTION

The wax/lipid layer in the external cuticle surface of all insects protect them from dehydration and pathologic infections (Lockey, 1988). These are also important as pheromones of the family- and genus-specific, but many kinds of termites also contain species-specific cuticular hydrocarbon components, suggesting that cuticular hydrocarbons (CHCs) of the termites can be used for species identification (Yang *et al*., 2013). These differences might be correlated with intercolonial aggressions (Jmhasly *et al*., 1998). Recent studies in this area suggest a genetic mechanism for cuticular hydrocarbons, but the research has not ruled out environmental effects on the composition of these substances in termites (Matsuura, 2001; Florane *et al*., 2004; Dronnet *et al*., 2006). The temperature and relative humidity on different times of a year may explicate variation in CHCs profile in termites (Haverty *et al*., 1996; Woodrow *et al*., 2000). These factors often cause no qualitative and minor quantitative alterations in the profiles, still, quantitative changes in nineteen CHCs of *Incisitermes minor* over time showed linear correlation (R^2=0.89) (Lewis *et al*., 2010). The genetics and environmental (abiotic and diet) factors may cause a significant variation in quantity of CHCs and how these variations affect function of the CHCs is not known.

Hydrocarbons affect permeability of the cuticle for dehydration and the absorption of insecticide; these also serve as recruitment stimulators as well as territorial markers (Howard and Blomquist, 1982; Blomquist *et al*., 1987). We are proposing a hypothesis in this paper that variations in CHCs may affect foraging and pesticides susceptibility of the termite.

MATERIALS AND METHODS

Collection of termites

The assorted workers of termites, *Odontotermes obesus* (Ramb.) (Termitidae: Isopetra) were collected from three place *viz*., Jhang, Faisalabad and Kamalia, in Punjab Province, Pakistan. The active epigeal mounds of this termite species were looked for and then fortnightly collection of workers from traps inserted into mound was done. These traps were corrugated cardboard rolled in plastic pipes which were inserted into active side of mounds.

Extraction and quantification of CHCs

The separated termite workers from card boards were frozen and subsequently dried in the desiccator held at room temperature until extraction. These workers (2 g)

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were then dipped in hexane in 10:1 (termites: ml hexane) ratio for one hour. Then the mixture was heated in a water bath for one hour (Ginzel *et al*., 2010). After cooling down, the supernatants were taken into a flask and hexane was evaporated by rotary evaporator. The remaining material was the hydrocarbons which was weighed by a digital weight balance (Wang *et al*., 2011). Different concentrations, *i.e.*, 150, 200 and 250µl of hydrocarbons mixture were made in hexane to detect hydrocarbons by using spectrophotometry technique and were quantified by using Beer's Formula; A= εcl.

Effect of temperature variations on cuticular hydrocarbons

The quantification of CHCs was determined at a range of temperatures for a given period of time, *i.e.*, 15, 20, 25, 30 and 35ºC for 3, 7, 10 and 14 days. For each temperature, a new set of workers were collected and kept for specific time intervals in three replications. Thereafter, hydrocarbons were extracted and quantified as described above.

Effect of extracted CHCs on the formation of galleries by the termites

Three concentrations (5, 10 and 15%) of extracted CHCs of the termites were made in acetone. The half of the soil of 20g in a Petri dish of 9 cm diameter was mixed with a concentration of CHCs and other half was treated with acetone only. This experiment was arranged in completely randomized design with three replications of each concentration for placing the termite workers in petri dishes. After releasing the termite workers, length of the tunnels were measured after 6, 12, 24, 48 and 72 h for each concentration and its control. The length of tunnel was plotted on cellophane paper and measured in mm by taking into account primary and secondary tunnels.

Susceptibility of individuals with high and low CHCs contents to pesticides

The termite workers during the months of low and high CHCs contents were collected and were exposed to field rate of imidacloprid (Mirage® 45 EC) in 20 gm soils in Petri dishes. To make the soil free from debris, it was sieved through a mesh 180-mm and oven dried at 100ºC for 24 h. One hundred termite workers were released in each Petri dish. Mortality of the termites was noted after 6, 12, 24, 48 and 72 h of the exposure. The Petri dishes containing termites were kept in an incubator run at 28±2ºC and 80±5% RH in three replications, each with a new set of termite workers.

Statistical analysis

CHCs extracted from termites from three different locations were compared in a year through ANOVA and means were compared using Tuckey's HSD test. Effect of temperature and exposure time on amount of CHCs, effect of CHCs application and exposure time on gallery length and percent mortality of termites with high and low CHCs contents at various post treatment intervals of imidacloprid exposure were analyzed using Generalized Linear Module in Minitab 16.0 (Minitab, Inc.).

RESULTS

CHCs contents of termites workers collected from three different places had non-significant difference among them. CHCs showed difference at various times of collection and highest contents were found in months of high ambient temperature starting from June through August. CHCs values ranged from 5 to 10mg/L in low temperature months while in high ambient temperature months the values were four times as high ranging from 40 to 48 mg/L. The highest value of 48mg/L was noted in the month of August after which there was a sharp decline in the amount of CHCs (Fig. 1).

The temperature had significant effect on contents of CHCs (df=4, F- ratio 33, $p<0.01$). The highest amount of CHCs was found at 35ºC when workers were kept for 7, 10 and 14 days (27.7, 27.5, 27.4 mg/L respectively), except at 3 days where contents were low (26 mg/L) at 35ºC. Termites kept at higher temperature near to the actual environment temperature in hotter months recorded an increase in CHCs, while those kept at lower temperatures showed lower amounts of CHCs. The interaction of days and temperature was non-significant ($p>0.05$). Amount of CHCs increased with rise in temperature from 15ºC to 35ºC, however, at each temperature, contents at different time intervals had non-significant difference among them (Fig. 2).

CHCs contents had also significant effect in the assemblage of termite workers and longer galleries were made in the highest concentration (15%) of CHCs. ANOVA showed that CHCs concentration (df=3, F-ratio 76, p=0.000), time (df=4, F-ratio 78, p=0.000) and the interaction of both these factors (df=12, F-ratio 20, p=0.000) showed significant effect on length of galleries formed inside the Petri dish. Termites exposed to higher concentrations for longer periods of time formed longer galleries and vice versa. The longest galleries (39.82 mm) were formed at 15% concentration after 72 h, while in its control counterpart, length of gallery was only 7.96 mm (Fig. 3).

ANOVA of termite types (based on high or low CHCs values), pesticide and time of exposure showed that termite types (df=1, F-ratio=6.70, p=0.01) and time of exposure (df=4, F-ratio=34.44, p=0.000) showed significant effect on percentage mortality. All the interaction terms were found non-significant (p>0.05). Termite type and time of exposure were compared using post hoc comparison and results showed that low CHC termites exposed for 72 h period recorded the highest percent mortality (73.9±3.63) and the lower percent mortalities (38.4±2.86 and 43.7±2.83) were recorded at 6 and 12 h, respectively. Percent mortality in high CHCs termites was significantly low (56.7±4.37) from those mortalities which was recorded in the termites of low CHCs contents (73.9±3.63) after 72 h of exposure (Table I).

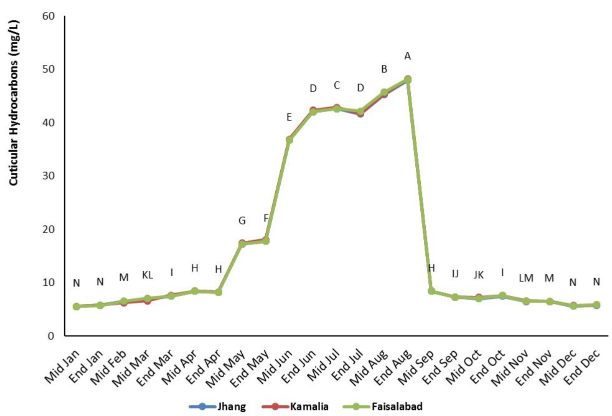

Fig. 1. Cuticular hydrocarbons (CHCs) contents of termite workers collected at various times of year 2013-2014 from three different places in Punjab, Pakistan.

Table I.- Percent mortality of termites with high and low CHCs contents at various post treatment intervals of imidacloprid exposure.

Exposure times (Hours)	Termite types	
	Low CHCs	**High CHCs**
6	38.4±2.86Da	23.9±2.58Cb
12	43.7±2.83Da	28.7±2.75Cb
24	58.1±3.04Ca	41.5±3.26Bb
48	66.1±2.88Ba	51.1±3.98Ab
72	73.9±3.63Aa	56.7±4.37Ab

Values are means±SE. Small letters represent difference between termite types and large letters show difference among exposure times.

DISCUSSION

Present studies have clearly demonstrated that temperature effected the amount of CHCs of the termite and the amount of hydrocarbons in the cuticle may depend upon the outside environment. The termites workers collected in time of higher temperatures resulted in an increase in CHC contents of the termite many fold than those at lower temperatures. It indicates the ability of the termite to regulate CHCs composition and contents and survive adverse conditions such as higher temperatures. As a result the CHCs were found to be in increased quantities. Previous studies have also reported CHC changed as a response to temperature fluctuations. Woodrow *et al*. (2000) reported an increase in high molecular weight hydrocarbon compounds in the cuticle, resulting an increase in the overall content of hydrocarbons in the cuticle under xeric conditions. Lockey (1988) and Noble-Nesbitt (1991) also proposed earlier that the new compounds formed might be used to regulate the viscosity of the cuticular lipid which is important when acclimatization is needed in response to changing environmental conditions.

To further investigate into this phenomenon, a second experiment was carried out in the laboratory which also resulted in the similar findings of increased CHC's in termites kept under higher temperatures similar to the temperature found in outside environment in the month of August when highest CHCs were recorded in the field. Irrespective of the time for which the termites were kept under different temperatures, the CHCs variation were found to be linear and constant. The similar observation was also reported by Howard *et al.* (1995) on adult sawtoothed beetles in which the major CHC modification was observed within 24 h.

The CHCs also act as communication cues for recruiting the nestmates for foraging and other activities which are not studied so far in termites, but are well documented in other social insects such as ants (Howard and Blomquist, 1982; Blomquist *et al*., 1987). In present study, higher CHCs concentrations applied for longer periods resulted in higher gallery lengths formed by termites as compared to their control counterparts. This suggests that the assemblage and behavioral patterns are modified as these CHCs may also act as communication signals. The high amount of CHCs may have led to larger assemblage and extensive foraging and this combined activity of the termites resulted in the longer gallery length in the present studies.

Though we could not find any report of low susceptibility of pesticides by the termite workers with higher CHCs, but high CHCs may interfere with the absorption of insecticides through the cuticle, thus resulting low termites' mortality. This study has practical implication. The month of August in Punjab, Pakistan, is characterized by high humidity and temperature (due to monsoon) and both these conditions favour termites'

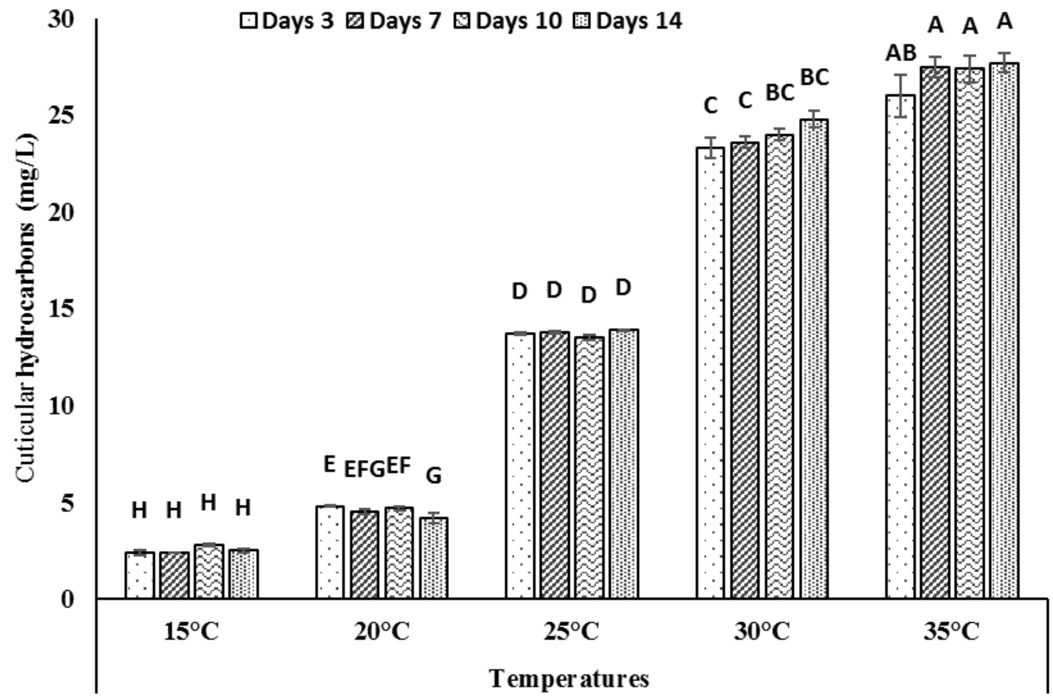

Fig. 2. Cuticular hydrocarbons in termite workers kept at different temperatures and times.

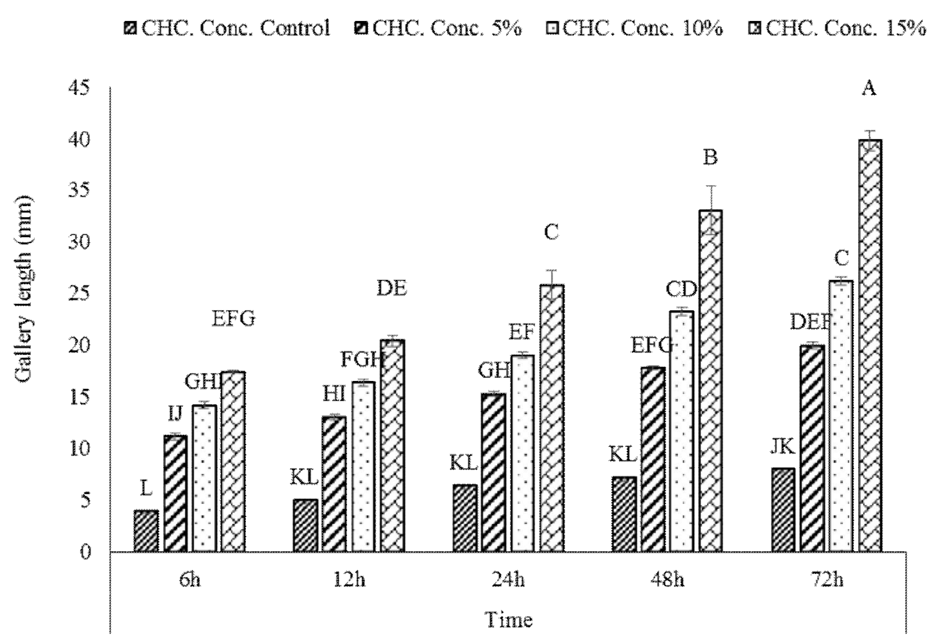

Fig. 3. Length of gallery formed in CHCs treated and untreated soils. CHC. Cuticular hydrocarbons, Conc. Concentration.

foraging and damage is noticeable in this month. The application of pesticides under high CHCs contents in hotter month of a year may not yield desired results of preventing the termites, thus, further studies are needed to find out ways to increase pesticides penetration in the months of high temperatures.

REFERENCES

Blomquist, G.J., Nelson, D.R. and DeRenobales, M., 1987. Chemistry, biochemistry and physiology of insect cuticular lipids. *Arch. Insect Biochem. Physiol*., **6**: 227-265.

Dronnet, S., Lohou, C., Christides, J.P. and Bagnères, A.G., 2006. Cuticular hydrocarbon composition reflects genetic relationship among colonies of the introduced termite *Reticulitermes santonensis* Feytaud. *J. chem. Ecol.*, **32**: 1027-1042.

Florane, C.B., Bland, J.M., Husseneder, C. and Raina, A.K., 2004. Diet-mediated inter-colonial aggression in the Formosan subterranean termite *Coptotermes formosanus*. *J. chem. Ecol.*, **30**: 2259-2574.

Ginzel, M.D., Blomquist, G.J. and Bagneres, A.G., 2010. *Insect hydrocarbons*. Cambridge University Press, Cambridge, U.K. pp. 375-377.

Haverty, M.I., Grace, J.K., Nelson, L.J. and Yamamoto, R.T., 1996. Intercaste, intercolony, and temporal variation in cuticular hydrocarbons of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). *J. chem. Ecol*., **22**: 1813-1834.

Howard, R.W. and Blomquist, G.J., 1982. Chemical ecology and biochemistry of insect hydrocarbons. *Annu. Rev. Ent*., **27**: 149-172.

Howard, R.W., Howard, C.D. and Colquhoun, S., 1995. Ontogenetic and environmentally induced changes in cuticular hydrocarbons of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae). *Ann. ent. Soc. Am*., **88**: 485-495.

Jmhasly, P., Brandl, R., Leuthold, R.H. and Kaib, M., 1998. Aggression and cuticular hydrocarbon profiles in a termite. *Zoology*, **101 Suppl. 1**:11**.**

Lewis, V.R., Nelson, L.J., Haverty, M.I. and Baldwin, J.A., 2010. Quantitative changes in hydrocarbons over time in fecal pellets of *Incisitermes minor* may predict whether colonies are alive or dead. *J. chem. Ecol.*, **36**: 1199-1206.

Lockey, H.K., 1988. Lipids of the insect cuticle: Origin, composition and function. *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.*, **89**: 595-645.

Matsuura, K., 2001. Nestmate recognition mediated by intestinal bacteria in a termite, *Reticulitermes speratus*. *Oikos*, **92**: 20-26.

Noble-Nesbitt, J., 1991. Cuticular permeability and its control. In: *Physiology of the insect epidermis* (ed. K. Binnington). CSIRO Publications, East Melbourne, Australia, pp. 252-283.

Wang, J.G., Zhan, X.H., Liang, J.R., Zhou, L.X., Lin, Y.S. and Jonathan, W.C.W., 2011. A novel method for the determination of total hydrocarbon in the hydrocarbon mixture contaminated soil. *J. Bioremed. Biodegrad.*, **34**: 355-361.

Woodrow, R.J., Grace, J.K., Nelson, L.J. and Haverty, M.I., 2000. Modification of cuticular hydrocarbons of *Cryptotermes brevis* (Isoptera: Kalotermitidae) in response to temperature and relative humidity. *Environ. Ent.*, **29**: 1100-1107.

Yang, J.J., Bao-Zhong, J.I., Liu, S.W., Cao, D.D., Zhang, X.W., Shin-Lin, J.I., Liu, J.J., Soleymaninejadian, E. and Wang, H.J., 2013. Research progress in cuticular hydrocarbons of termites. *Acta entomol. Sin*., **56**: 1480-1488.