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### RESEARCH ARTICLE

#### PHYSICO-CHEMICAL PROPERTIES OF TERMITE CELLULASES (INSECTA: ISOPTERA) BASED ON THE TROPHIC GROUP (DALOA, COTE D'IVOIRE)

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#### Abstract

The distribution of termites according to trophic groups in agrosystems, causes damage leading to a decrease in yield of crops. This results in growing food insecurity for the population. Many control methods used against these pests have shown their inadequacy. The understanding of the functioning of digestive enzymes is therefore necessary. The present study aims to compare the physico-chemical properties of termite cellulases as a function of trophic groups. Four enzymatic extracts of humivorous (*Cubitermes fungifaber*) and xylophagous termites (*Amitermes guineensis*, *Nasutitermes latifrons*, *Microcerotermes fuscotibialis*) were used. The results show an optimum difference in temperature of hydrolysis within xylophage cellulases, although they are all mesophilic, acidic with optimum hydrolysis pH of 5.6 and better expressed in 20 mM sodium acetate buffer. In addition, the cellulase activities of *C. fungifaber* and *A. guineensis* are maximum at 60 °C while those of *N. latifrons* and *M. fuscotibialis* are at 55 °C respectively. As for relative activities, they are different among the three xylophagous species. *N. latifrons* has the highest relative cellulase activity. This result reflects the voracious behavior of this one on cocoa trees.

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#### Introduction:-

Termites are insects that influence soil properties (Dosso and Kone, 2016). They cause a lot of damage on the plantations thus leading to food insecurity for the populations (Coulibaly et al., 2014; Sane et al., 2016; Tra Bi et al., 2019). However, traditional methods (use of cakes as an insect repellent, destruction of nests), chemical (use of fipronil in sugarcane plantations, imidacloprid, very toxic furadan, Dursban, pychlorax on mango trees) (Mampouya, 1997; Akpessa et al., 2008) and biological (use of dead wood in fruit trees, entomopathogens advocated by Han and Ndiaye (1996)); plant extracts have been used to stop termite damage. But, these showed their limit. The establishment of more effective means of struggle is therefore necessary. Indeed, the strong expansion of termites as pests of cultivated plants is due to their great ability to degrade the constituents of wood (polysaccharides, lignin, tannins, etc.) thanks to digestive enzymes such as the cellulases they possess (Kumari et al.,

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2006). So do these enzymes work within the same trophic group in the same way? In other words, do these cellulases have the same physicochemical properties? In addition, the work, led by Sea et al. (2006) and Blei et al. (2018) on the biochemical characterization of cellulases of workers and great soldiers of the termite *Macrotermes subhyalinus* (Termitidae, Macrotermitinae) belonging to the group of mushroom growers, showed different behaviors. The present study aims to understand the digestive function of termites collected from plantations (cocoa, coffee and teak plantation) to better understand the resistance of these organisms to the means of control advocated by predecessors. Specifically, the physicochemical properties of the cellulase activities of humivorous (*Cubitermes fungifaber*) and xylophagous termites (*Amitermes guineensis*, *Nasutitermes latifrons* and *Microcerotermes fuscotibialis*) will be compared.

## **Material and Methods:-**

### **Biological material:**

The biological material consists of termite species (*A. guineensis*, *C. fungifaber*, *N. latifrons* and *M. fuscotibialis*). Termites were collected in cocoa, coffee and teak plantations in Daloa (Côte d'Ivoire).

### **Sampling technique:**

Termites were first harvested in dead woods and soil with equipment (such as daba, machete) and kept in perforated boxes to let air in to keep them alive. Then, some termites of each species were kept in labeled eppendoffs containing 70% alcohol for identification. And other samples were brought to the laboratory to be stored at -20 °C in a freezer for analysis of their enzyme equipment.

### **Identification of termite species:**

The identification of different species of termites collected, was carried out using a binocular loupe (Leica brand). This technique was based preferentially on the caste of soldiers and that of workers for termites without soldiers. Several identification keys or articles were consulted for proper identification (Sand, 1972, 1998).

### **Technique for obtaining enzymatic extracts:**

Five hundred and fifty (550) workers of various termite species were washed with distilled water and dewatered on whatmann paper No.1. These samples were ground in a porcelain mortar containing 30 ml of NaCl (0.9%, w / v). The ground material obtained was centrifuged at 13,750 rpm for 30 minutes at a temperature of 4 °C in a 5427R centrifuge. The obtained supernatant constituted the enzymatic crude extract of the workers (*A. guineensis*, *C. fungifaber*, *N. latifrons* and *M. fuscotibialis*).

### **Measurement of cellulase activity:**

For the measurement of cellulase activity, the dosage of reducing sugars was carried out by the method of Bernfeld (1955) using 3,5-dinitrosalicylic acid (DNS). The reaction medium consisting of 80 µl of 20 mM acetate buffer pH 5.0, 100 µl of enzyme solution and 200 µl of substrate (Carboxymethylcellulose, 0.5%, w/v) was used. This reaction medium was incubated in a water bath at 37 °C. for 30 minutes. Then, 300 µl of a DNS solution is added to stop the enzymatic reaction. It was then homogenized and heated on a steam bath for 5 minutes and cooled for 10 minutes at room temperature (25 °C). Absorbance was measured at 540 nm spectrophotometer (Gilson) against a control (containing all products except the enzyme solution) after adding 2 ml of distilled water. This absorbance was then converted into micromoles of reducing sugars by means of a calibration line obtained using a glucose solution (2 mg / ml).

### **Determination of physicochemical parameters:**

#### **Optimum pH of hydrolysis:**

The optimum pH of hydrolysis was determined in 20 mM sodium acetate buffers (pH 3.6 to 5.6) and 20 mM sodium phosphate (pH 5.6 to 8.0). The corresponding enzymatic activities were determined under standard conditions.

#### **pH stability zone**

Stability of the enzyme at pH was investigated by pre-incubating at room temperature (25 °C) for 2 h at different pH (3.6 to 8.0) in the above buffers. Residual relative activities were determined under standard conditions.

**Optimum hydrolysis temperature:**

The influence of temperature on cellulase activity was studied in 20 mM acetate buffer at pH 5.6 at temperatures ranging from 35 to 80 °C. Cellulase activity was determined under standard conditions.

**Energie d'activation (Ea) et du coefficient de température (Q<sub>10</sub>):**

To calculate the value of the temperature coefficient (Q<sub>10</sub>), the mathematical relationship of equation 1 was used (Arrhenius, 1889).

$$Q_{10} = X_{10} / X \quad (1)$$

X<sub>10</sub> = Optical Density at 50 °C (the O.D. at 50 °C)

X = Optical Density at 40 °C (the O.D. at 40 °C)

The Arrhenius graph (1889) for determining activation energy was obtained in the temperature range between 35 and 60 °C for cellulase activity. Activation energy (Ea) was determined from the mathematical relationship n°2.

$$Ea = -2,303 \times a \times R \quad (2)$$

With R = 8.31 kJ / mol; a = Slope (from the Arrhenius graph, Log (% activity) = a x (1 / T) + b).

**Statistical Analysis:-**

The data were encoded using the Excel 2013 spreadsheet. The different analyzes were done using PAST 3.14 software and the data processing was based on the analysis of variance and multiple average of intra and interspecific cellulase activities of termites. Shapiro-Wilk tests were applied to verify the normality of the data. Thus, analysis of variance (ANOVA) made it possible to determine the difference of the physicochemical properties of these termites. Subsequently, the Tukey test at the threshold of  $p < 0.05$  made it possible to separate the different groups obtained.

**Results and Discussion:-**

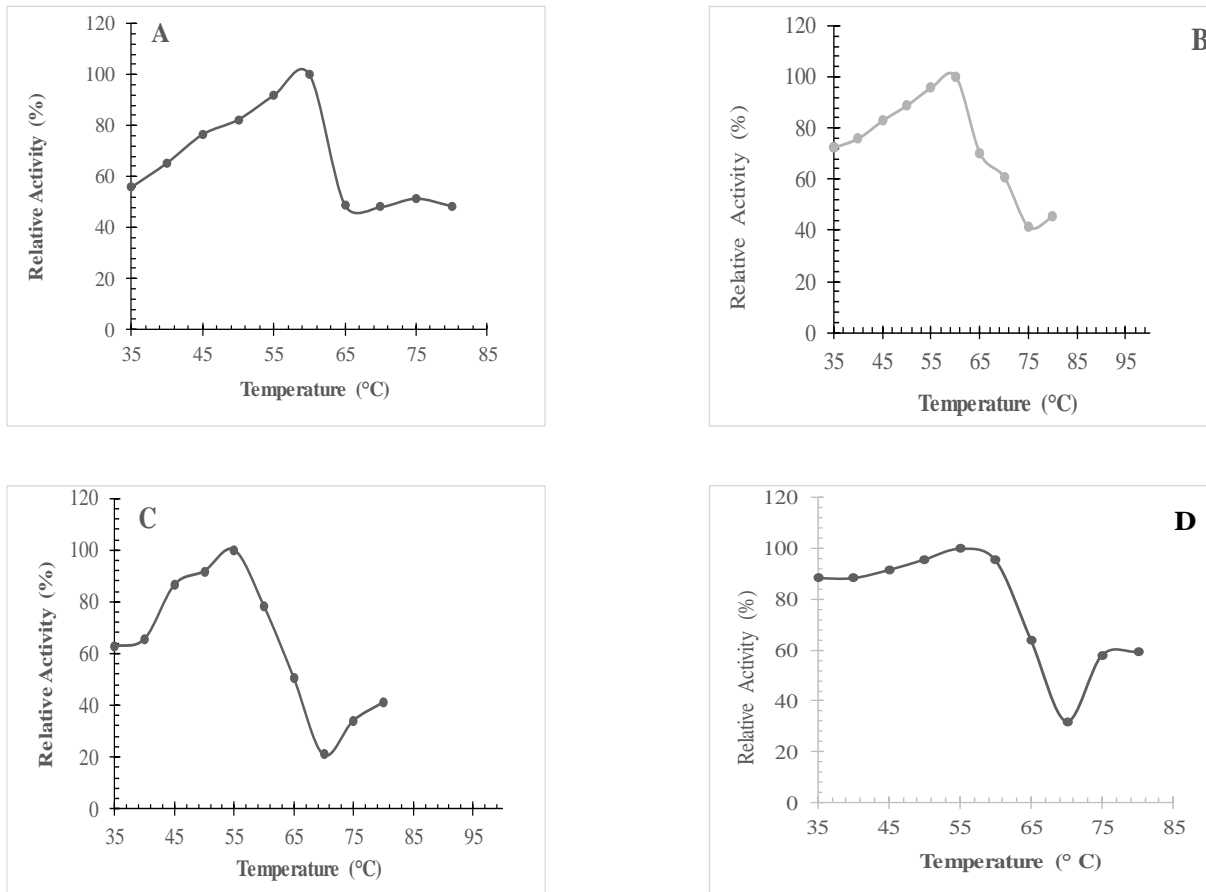
The optimal pH of hydrolysis of the cellulase activities of the various species of termites studied is 5.6. The activity has a significant difference. This difference is observed between *A. guineensis* and *C. fungifaber* with a probability of 0.01787 (Table 1). This activity is better expressed in the sodium acetate buffer. The stability zone at pH is between 4.6 and 7. The activity shows a significant difference. This difference is observed between *M. fuscotibialis* and *C. fungifaber*, which records a probability of 0.0161 (Table 1). The relative activities of termites are different from each other at optimal hydrolysis temperatures (Table 1). They are respectively  $0.066 \pm 0.001$ ,  $0.031 \pm 0.002$ ,  $0.024 \pm 0.008$  and  $0.013 \pm 0.000$   $\mu\text{mol} / \text{min}$  for *N. latifrons*, *A. guineensis*, *C. fungifaber* and *M. fuscotibialis* (Table 1). The highest activity is obtained with *N. latifrons* termite at pH and optimal hydrolysis temperature.

Optimum pH hydrolysis of *C. fungifaber*, *N. latifrons*, *M. fuscotibialis* and *A. guineensis* species are identical and moderately acidic (pH 5.6). pH acidity is a major advantage for the bleaching of acid or neutral pH papers in industries (Viikari *et al.*, 1994). Studies by Jun *et al.* (2009) describe optimal activity at pH between 5 and 6 for mutants NU 6 and *Trichoderma reesei* Rut C-30. This corroborates with the results of this study. On the other hand, optimum pH values of 4.0 and 5.0, respectively, are widespread in cellulases of various microorganisms such as *T. reesei* (Krishna *et al.*, 2000). Similarly, the pH of the results obtained are in the range of the optimal pH of hydrolysis of the enzymes  $\beta$ -glucosidases and *Sclerotinia sclerotium* endoglucosidases located between 4 and 5 (Saloua *et al.*, 2000). Camilo *et al.* (2016) found the optimal enzymatic activity of cellulases of some species at pH 5. Other results of the work of Abdul-Hadi *et al.* (2016) have an optimal pH of 6 with *T. reesei* cellulase. All these results show that there is a difference between cellulases at the optimum pH of hydrolysis. The activities are best expressed with sodium acetate buffer.

**Table 1:-** Optimum pH of hydrolysis and pH stability zone.

Trophic group	Humivore	Xylophage		
Enzymatic extract of termites	<i>C. fungifaber</i>	<i>N. latifrons</i>	<i>M. fuscotibialis</i>	<i>A. guineensis</i>
optimum pH of hydrolysis	5,6	5,6	5,6	5,6
Relative activity ( $\mu\text{mol /min}$ )	0,024 $\pm$ 0,001	0,071 $\pm$ 0,001	0,012 $\pm$ 0,001	0,026 $\pm$ 0,001
pH stability zone	5,0 - 6,0	4,6 - 7,0	4,6 - 6,6	4,6 - 5,0
Optimum hydrolysis temperature ( °C)	60	55	55	60
Relative activity ( $\mu\text{mol /min}$ )	0,024 $\pm$ 0,008	0,066 $\pm$ 0,001	0,013 $\pm$ 0,000	0,031 $\pm$ 0,002

The optimal temperature of hydrolysis of the cellulase activity of termites *C. fungifaber*, *A. guineensis* is 60 °C while that of *N. latifrons* and *M. fuscotibialis* is 55 °C with carboxymethylcellulose as a substrate in the acetate buffer 20 mM pH 5.0. At this temperature, the cellulase activity is maintained at more than 80% (Fig. 1). In addition, 80 to 100% of the cellulase activity is preserved at temperatures between 45 and 60 °C. The activity decreases to below 50% when the temperature rises above 70 °C. The variation of the optimum cellulase hydrolysis temperatures obtained at the level of the xylophagous termites (60 °C for *A. guineensis*, 55 °C for *N. latifrons* and *M. fuscotibialis*) confirms their differences in cellulase relative activities. This reflects their voracity on plants grown in Côte d'Ivoire (Han et al., 1998; Coulibaly et al., 2014; Tra Bi et al., 2019; this could be explained by the plasticity of certain termite species). characterized by their distribution and abundance in habitats based on edaphic characteristics (Sarr et al., 2005 ; Sane et al., 2016), but overall, cellulases have high maximum temperatures relative to Other enzymatic systems: Studies by Saloua et al (2000) found an optimum temperature of 55 and 60 °C for  $\beta$ -glucosidase and an endoglucanase of the *S. sclerotium* fungus. The carboxymethylcellulase activity produced by *T. reesei* and *Trichoderma* sp, respectively, was 60 °C. (Hind, 2013) These results are identical to those obtained in this study for the species *C. fungifaber*, *A. guineensis*. However, the optimal temperature of the work of Lekchiri et al. (2013) on the cellulase activities of *Fusarium oxysporium* sp *Albedinis* is 50 °C. The study conducted by Jun et al. (2009) showed an optimal temperature of 50 °C with the carboxymethylcellulase produced by the NU6 mutant of *T. reesei* Rut C-30. These results are lower than the value obtained in this study. In addition, the maximum temperature of fungal cellulases varies between 40 and 70 °C. On the other hand, that of bacteria is between 50 and 100 °C, corresponding to the optimal temperature range for this study (Ando et al., 2002). In addition, all the termite cellulases studied are mesophilic. This mesophilic property is an important asset for the agri-food, paper and biotechnology industries. Some industries use high temperature processes (Zamost et al., 1991). The increase in temperature has been reported to contribute to the degradation of lignin and cellulose by thermophilic enzymes (Bhalla et al., 2013).



**Fig 1:-**Influence of temperature on cellulase activity of termites.  
 A: *C. fungifaber* ; B: *A. guineensis* ; C: *M. fuscotibialis* ; D: *N. latifrons*

According to the law of arhenius, the activation energy is in direct relation with the effect of the temperature. This energy is obtained by graphing the logarithm of the percentages of activities as a function of the inverse temperature ( $1/T$ ) taking into account the data of the acceleration part of the temperature curve (Fig. 1 and 2). Activation energies of the cellulite activities of termites are respectively 38.5 KJ / mol for *C. fungifaber*; 38.34 KJ / mol for *N. latifrons*; 38.38 KJ / mol for *A. guineensis* and 38.47 KJ /mol for *M. fuscotibialis* with respective temperature coefficients ( $Q_{10}$ ) of 1.37; 1.09; 1.7 and 1.41 (Fig. 2).

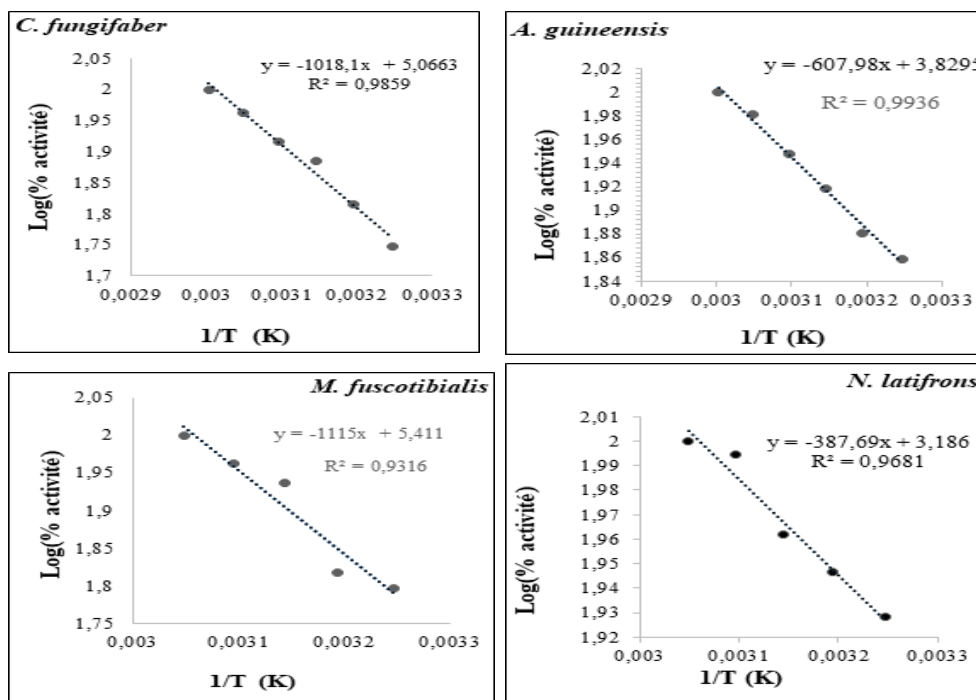


Fig 2:-Activation energy of cellulase activities of termites

### Conclusion:-

The study conducted on the physicochemical properties of cellulases of xylophagous (*A. guineensis*, *N. latifrons* and *M. fuscotibialis*) and humivorous (*C. fungifaber*) termite species showed a difference between the two groups. The temperature difference observed in the xylophages reflects the voracious behavior of this group on cultivated plants. The characterization of enzymes responsible for the degradation of constituents of plant matter such as cellulases could inspire biotechnologists to consider the strengthening of existing control resources and / or the development of new control methods for efficient management of these organisms pests.

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