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2 **Inhibitors of cellulase activities according to**
3 **the trophic group of termites (Insecta: Isoptera)**
4 **from Daloa (Côte d'Ivoire)**
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6

7 **ABSTRACT**
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The presence of termites in the cocoa plantations and quarries of Côte d'Ivoire poses a threat to the producers of this sector. Producer yields are insufficient to cover the strong market demand. This situation leads to food insecurity for the population. Knowledge of the specific inhibitory molecules of digestive enzymes of termites is necessary to enhance the effectiveness of insecticides to optimize crop production. The present study aims to characterize termite cellulases according to the trophic group. Specifically, the influence of chemical agents on the cellulase activities of four humivorous (*Cubitermes fungifaber*) and xylophagous termites (*Nasutitermes latifrons*, *Microcerotermes fuscotibialis* and *Amitermes guineensis*) collected in Daloa during the October period was investigated. December. Thus, the cellulase activities were measured by the spectrophotometric method in the absence and in the presence of the concentrations of 1 and 5 mM of various chemical agents. The chemical agents used behave differently on cellulase activities. Thus, Cu^{2+} , Pb^{2+} and EDTA more than 90% inhibit the cellulase activity of *M. fuscotibialis* at concentrations of 1 and 5 mM, respectively, indicating the presence of a metalloprotein. On the other hand, that of the other two xylophagous species is slightly inhibited. In addition, the cellulase activity of *C. fungifaber* is inhibited at the two respective concentrations by Cu^{2+} at about 70%. In conclusion, Cu^{2+} , pb^{2+} and EDTA can be used in the formulation of some specific insecticides against humivorous and xylophagous termites.

9
10 *Keywords: Chemical agents; Cellulases; Termites; Cultures; Côte d'Ivoire.*
11

12 **1. INTRODUCTION**

13 In terrestrial ecosystems, termites have important functions. Thus, numerous works carried
14 out in Ivory Coast showed the damage caused by these insects on the oil palm [1], the
15 rubber tree [2], in the mango orchards [3] and the cacao tree [4].

16 The strong expansion of termites as pests of cultivated plants is due to their great ability to
17 degrade the constituents of wood (polysaccharides, lignin, tannins, etc.) thanks to the
18 digestive enzymes they possess, notably cellulases, which are responsible for the
19 degradation. cellulose [5]. Studies conducted by Blei et al. On the determination of the
20 physicochemical properties of cellulases of soldiers of *Macrotermes subyalinus* [6, 7, 8] and
21 termite workers according to their trophic group (publication in progress) have shown that
22 Behave differently Faced with this situation, strengthening the efficacy of insecticides is
23 therefore necessary to guarantee crop production.

24 The present study aims to characterize termite cellulases according to the trophic group.
25 More specifically, it will be necessary to determine the chemical agents capable of inhibiting
26 the cellulase activities of four species of humivorous (*Cubitermes fungifaber*) and
27 xylophagous termites (*Nasutitermes latifrons*, *Microcerotermes fuscotibialis* and *Amitermes*
28 *guineensis*) collected at Daloa in order to know the inhibitors.

29 **2. MATERIAL AND METHODS**

30 ***2.1 Biological material***

31 The biological material consists of the species of Humivorous termites (*Cubitermes*
32 *fungifaber*) and xylophagous (*Amitermes guineensis*, *Nasutitermes latifrons* *Microcerotermes*
33 *fuscotibialis*) collected in plantations of cacao, coffee and teak of Daloa (Côte d'Ivoire).

34 ***2.2 Methods***

35 **2.2.1 Sampling technique**

36 Termites were first harvested from dead woods and soil with equipment (such as
37 daba, machete) and kept in perforated boxes to let air through to keep them alive.
38 Then, some termites of each species were kept in labeled eppendoffs containing 70%
39 alcohol to identify them. The identification of different species of termites collected,
40 was carried out using a binocular loupe. Several manuals have been used to identify
41 them [9]. And other termite samples were brought to the laboratory to be stored at -20
42 ° C in a freezer for analysis of their enzyme equipment.

43 **2.2.2 Technique for obtaining enzymatic crude extracts**

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

50 **2.2.3 Measurement of cellulase activity**

51 For the measurement of cellulase activity, the dosage of reducing sugars was carried out by
52 the Bernfeld method [9] using 3,5-dinitrosalicylic acid (DNS). The reaction medium
53 consisting of 80 µl of 20 mM acetate buffer pH 5.0, 100 µl of enzymatic solution and 200 µl
54 of substrate (Carboxymethylcellulose, 0.5%, w / v) was used. This reaction medium was
55 incubated in a water bath at 37 °C. for 30 minutes. Then, 300 µl of a DNS solution is added
56 to stop the enzymatic reaction. It was then homogenized and heated on a steam bath for 5
57 minutes and then cooled for 10 minutes at room temperature (25 °C). Absorbance was
58 measured at 540 nm spectrophotometer (Gilson) against a control (containing all products
59 except the enzyme solution) after adding 2 ml of distilled water. This absorbance was then
60 converted into micromoles of reducing sugars by means of a calibration line obtained using a
61 glucose solution (2 mg / ml).

62 **2.2.4 Influence of chemical agents on enzymatic activities**

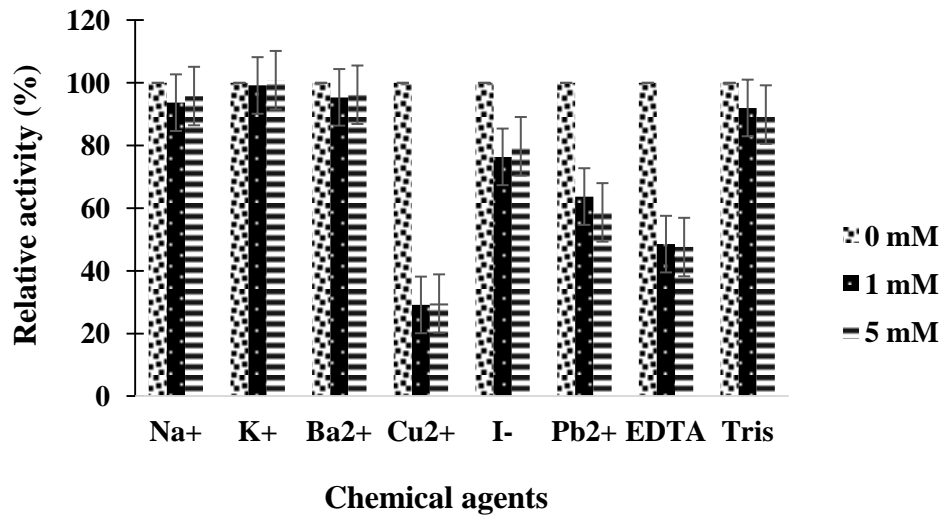
63 The effect of chemical agents on enzyme activity was studied by pre-incubating the
64 enzymatic crude extract of each termite species for 2 hours at room temperature (25 °C) in
65 the presence of different chemical agents such as salts of potassium chloride (KCl), sodium
66 chloride (NaCl), barium chloride (BaCl₂), copper sulphate (CuSO₄), potassium iodide (KI),
67 lead acetate (pb(C₂H₃O₂)₂), ethylene diamine tetra acetate (EDTA) and hydroxymethylamino
68 methane (tris), at concentrations of 1mM and 5mM, respectively. Cellulase related activities
69 were measured under standard conditions.

70 **3. RESULTS AND DISCUSSION**

71 **3.1 RESULTS**

72 The results of FIGS. 1A, 1B, 1C, and 1D show the sensitivity of the enzymatic activity in the
73 presence of some metal ions (Na⁺, K⁺, Ba²⁺, Cu²⁺, Pb²⁺, I⁻), EDTA and Tris. For the 1 mM
74 and 5 mM concentrations, the Na⁺, K⁺, Ba²⁺ and Tris agents have virtually no effect on the
75 cellulase activity of *C. fungifaber*, *A. guineensis* and *N. latifrons* (Fig. 1, 3 and 4). However,
76 at the concentration of 5 mM, Tris and Ba²⁺ ion activate the cellulase activities of termites *M.*
77 *fuscotibialis* and *N. latifrons* respectively at 31 and 11% (Figures 2 and 4). In addition, Cu²⁺
78 and EDTA are present as inhibitors (Figures 1, 2, 3 and 4) at concentrations of 1 and 5 mM,
79 respectively. However, the cuprous ion (Cu²⁺) inhibits the cellulase activity of *M. fuscotibialis*
80 by more than 95% compared to the metal ions used (Fig.2). In addition to the Cu²⁺ ion and
81 EDTA, the iodide I⁻ ion inhibits the cellulase activities of *C. fungifaber*, *M. fuscotibialis* and *N.*
82 *latifrons* (Fig. 1, 2 and 4) while it has no effect on that of *A. guineensis* (Fig. 3). In addition,
83 the Pb²⁺ ion inhibits respectively the cellulase activities of *C. fungifaber*, *M. fuscotibialis* and
84 *N. latifrons* at 42,70 and 36% at the concentration of 5 mM (Fig. 4). On the other hand, it
85 activates by 8% the cellulase activity of *A. guineensis* at concentrations of 1 and 5 mM
86 respectively (Fig. 3).

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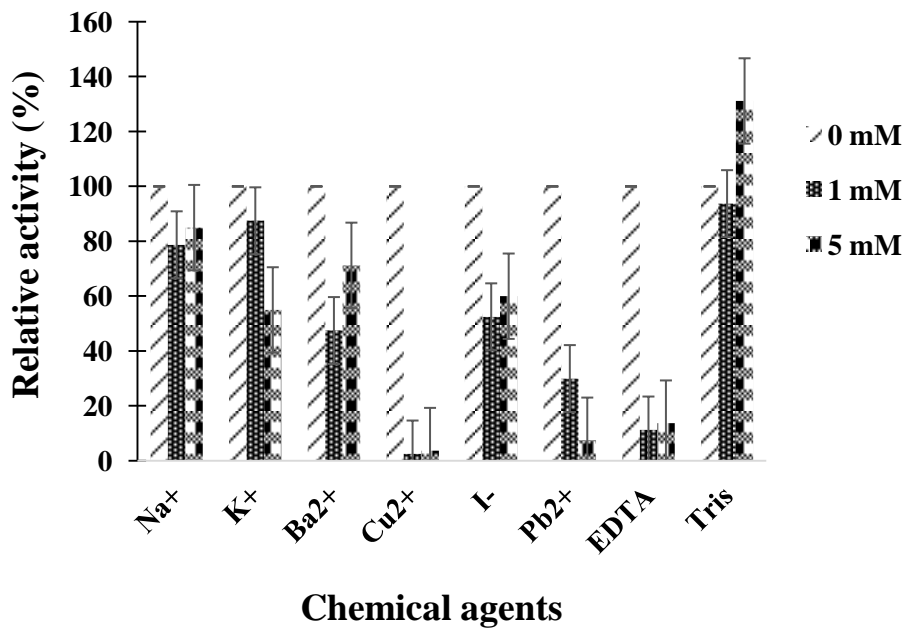
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89 . **Fig.1:** Cellulase relative activity of termite *C. fungifaber* as a function of the
 90 concentration of chemical agents.

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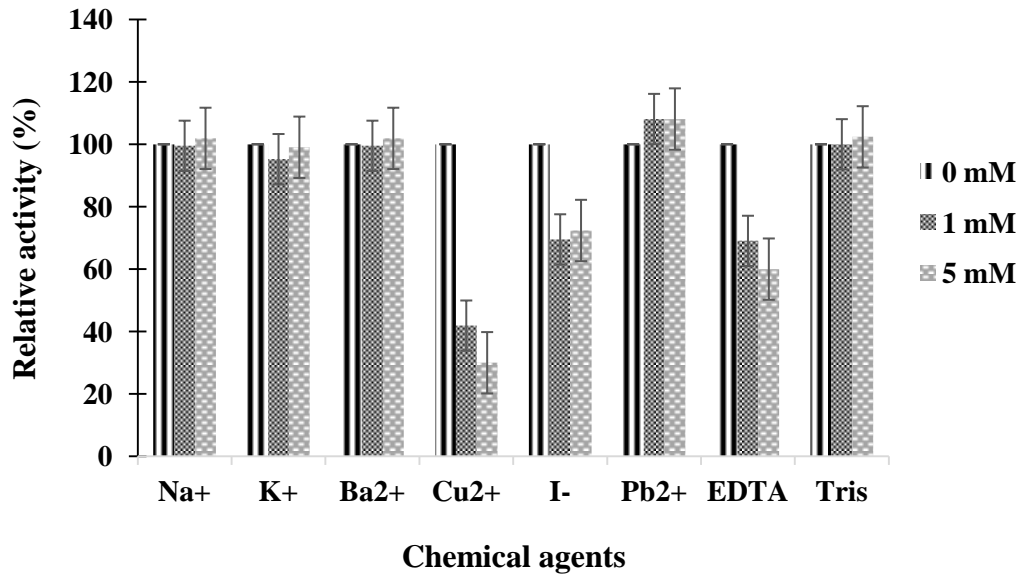
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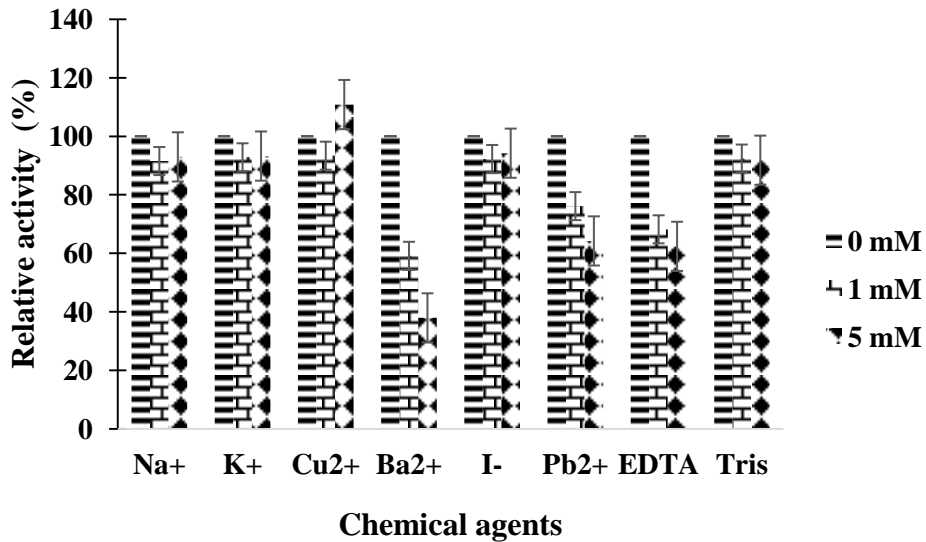
95 Fig.2: Relative cellulase activity of termite *M. fuscotibialis* as a function of the
 96 concentration of the chemical agents.
 97

98



99

100 Fig.3: Cellulase relative activity of termite *A. guineensis* as a function of the
 101 concentration of chemical agents.



102

103 **Fig.4: Cellulase relative activity of *N. latifrons* termite as a function of the**
104 **concentration of chemical agents.**

105
106 **3.2 DISCUSSION**

107 Sodium and potassium chloride salts influence the change in enzymatic activity, certainly
108 through interaction with regulatory sites [10]. According to Roy **et al.**, [11]. Na⁺ and K⁺ ions
109 have an effect on enzymatic activity due to changes in electrostatic binding that would affect
110 the tertiary structure of the enzyme. Comparison of enzymatic activity with other studies has
111 shown that the endoglucanase activity of *Aspergillus flavus* increases with increasing
112 concentration of Na⁺ and K⁺ cations [12]. However, this activity is strongly influenced by the
113 presence of the K⁺ ion with a concentration of 1 mM (85% of the activity). In the case of this
114 study, the K⁺ and Na⁺ ions have practically no effect on the activity. A study of bacterial
115 cellobiohydrolases reported a slight improvement in activity with Ba²⁺ [13; 14], and a fungal
116 cellulase was inhibited by Ba²⁺. For their part, Zhu **et al.** [15] showed that the Ba²⁺ ion could
117 lead to a moderate increase in the activity of a *Geobacillus* esterase and deep with a
118 concentration of 10 mM. All these results are in the same direction as those reported in this
119 study with the different species studied because these same effects of barium were found.
120 This behavior of the Ba²⁺ ion on the cellulase activity could be due to the composition of the
121 amino acids or to the presence of certain ions in the catalytic site of the enzyme. Inhibition of
122 cellulase activity by Cu²⁺ ion are consistent with those obtained by several authors. Thus,
123 Roy **et al.** [16] have shown in previous studies that Cu²⁺ has significant inhibition on
124 endoglucanases in *Myceliophthora thermophila* D-14 (ATCC48104A). Similarly, Deb **et al.**
125 [17] show inhibition of enzymatic activities in *Bacillus amyloliquefaciens* P-001 by a number
126 of metal ions, including Cu²⁺ copper ion. According to these authors, this divalent ion
127 behaves as a non-competitive inhibitor of enzymatic activity. Copper does not attach to the
128 active site as competitive inhibitors. It is rather related to a side group of the enzyme thus
129 modifying the structure of the enzyme. Also, the way it folds changes the active site [18]. In

130 addition, the indirect reduction of enzymatic activity following the interaction of the toxic part
131 of copper with the microorganisms affects the enzymatic production [19]. Thus, the metals
132 can bind to the substrate or react with the substrate enzyme complex [20]. Inhibition of lead
133 is thought to be due to the presence and fusion between lead and thiol groups of the enzyme
134 [21, 22], since lead increases the activity of other enzymes. Thus, lead varies the
135 characteristics of these enzymes or stop the activity of their inhibitors [21]. This is the case of
136 the species *A. guineensis* whose activity increases in the presence of lead. This is in
137 agreement with the studies of Seregin & Ivanov [21]. Lead inhibits enzymatic activities as a
138 whole and achieves an inactivation constant of between 10^{-5} and 2×10^{-4} M, which means
139 that 50% of enzyme activities are inhibited in this concentration range [21]. Pb^{2+} is therefore
140 a potent inhibitor [10]. These results corroborate with the results obtained for *C. fungifaber*,
141 *N. latifons* and *M. fucotibialis* species. The inhibition of enzymatic activity by EDTA in the
142 four species studied is explained by the complexation of certain metal ions necessary for the
143 activation and stabilization of the enzyme [23]. These enzymes are metalloproteinic in
144 nature. The low activity suggests that the metal ion has a very high affinity for the enzyme or
145 that the ion is difficult to access in the assay because of steric constraints or amino acid
146 residues because the inhibitory effect of this compound depends on the relative stability of
147 the EDTA-ion complex compared to that of the ion-enzyme complex. Previous studies on
148 endoglucanases, Lee et al. [24] on *Bacillus amyloliquefaciens* DL-3 showed activity inhibition
149 with the presence of EDTA. These results are identical to this study. On the other hand,
150 other studies have shown that at concentrations of 1 and 5 mM, EDTA had no effect on the
151 amylase activity of Archaea. Therefore, this result deduces that the enzyme is not a
152 metalloprotein [25]. Which is not consistent with that of this study.

153 **4. CONCLUSION**

154 The divalent ion Cu^{2+} and the EDTA ion chelator are presented as inhibitors of the cellulase
155 activities of the 4 species studied. Moreover, the Pb^{2+} ion inhibits the cellulase activity of
156 *Microcerotermis fuscotibialis*. The Cu^{2+} and Pb^{2+} ions as well as the EDTA ion chelator can be

157 used in the formulation of certain specific insecticides for strengthening the fight against
158 humivorous and xylophagous termites.

159 **COMPETING INTERESTS**

160 Authors have declared that no competing interests exist.

161 **AUTHORS' CONTRIBUTIONS**

162 All authors read and approved the final manuscript.

163 **REFERENCES**

- 164 1 Han SH, Ndiaye AB. Damage caused by termites (Isoptera) on fruit trees in the
165 region of Dakar (Senegal). *Actes coll, Social insects*.1996; 10: 111-11. French.
- 166 2 Tahiri A, Mangué JJ. Strategies for attacking Hevea seedlings (Hevea
167 brasiliensis Muell) by termites and compared effect of two insecticides used for their
168 protection in low Côte d'Ivoire, *Sciences & Nature*, 2007; 4 (1): 45-55. French.
- 169 3 Coulibaly T, Akpesse AAM, Yapi A, Zirihi GN, Kouassi KP. Termite damage in
170 mango nurseries in northern Côte d'Ivoire (Korhogo) and control trial using aqueous
171 extracts of plants. *Journal of Animal & Plant Sciences*. 2014; 22(3): 3455-3468.
172 French.
- 173 4 Tra Bi CS, Blei S H, Coulibaly T, Soro S, Tano Y. attacks modalities and biogenics
174 structures of termites (insecta: isoptera) cocoa pests (theobroma cacao L.) (Oumé:
175 Côte d'Ivoire). *International Journal of Agriculture Sciences*. 2019; 11(16) : 8902-
176 8907.
- 177 5 Kumari V, Aasen I, Flytch D, Williams SCR, Sharma T. Neural correlates of
178 adjunctive rivastigmine treatment to antipsychotics in schizophrenia: a randomized,
179 placebo controlled, double blind fMRI study. *NeuroImage*. 2006; 29(2): 545-556.
- 180 6 Blei SH, Soro YR, Dabonne S, Kouame P L. A nouvel polysaccharide with endo-
181 beta- D xylanase and endo- beta - D glucanase activity in the gut of the major
182 soldier of the termite *Macrotermes subhyalinus*. *J. Anim. Plant. Sci*. 2010; 8(1): 912-
183 926.

- 184 7 Blei SH, Dabonne S, Soro YR., Kouame P L. Purification and characterization of an
185 endo- beta- D- xylanase from major soldier salivary glands of the termite
186 *Macrotermes subhyalinus*. With dual activity against carboxymethylcellulose. *J.*
187 *Entomol. Nematol.* 2011; 3 (1): 1-13.
- 188 8 Blei SH, Tra BC S, Kone FMT, Faulet MB, Beugre G.A M, Soumaila D, Niamke LS,
189 Kouamé LP. Effect of Hydroquinone and Naphtoquinone on the Purified Bifunctional
190 Endoxylanase from the Salivary Glands of the Major Soldier of Termite *Macrotermes*
191 *subhyalinus* (Insecta: Isoptera). *International Journal of Science and Research*,
192 2018; 7(4): 1006-1008.
- 193 9 Bernfeld P. Amylase alpha and beta. (Assay method), in *Methods in enzymology*
194 1.S. P. Colswick and N.O. Kaplan., Edition *Academic Press* (New-York). 1955; pp
195 149-154.
- 196 10 Singh A., Agrawal A. K., Abidi A B. & Darmwal N. S. General and kinetic properties
197 of endoglucanase from *Aspergillus niger*. *FEMS Microbiology Letters*. 1990; 71(1-2):
198 221-224.
- 199 11 Roy S K., Dey S K., Raha S K. & Chakrabarty S L. Purification and properties of an
200 extracellular endoglucanase from *Myceliophthora thermophila* D-14 (ATCC 48104).
201 *Journal of General Microbiology*. 1990; 136: 1967-1971.
- 202 12 Ajayi A A., Adejuwon AO, Awojobi OK, Olutiola PO. Effect of cations and chemicals
203 on the activity of partially purified cellulase from tomato (*Lycopersicon esculentum*
204 *Mill*) fruits deteriorated by *Aspergillus flavus*. *Pak. J. Nutr.* 2007; 6(2) : 198-200.
- 205 13 Kim DW, Jang YH, Kim C S. Effect of metal ions on the degradation and
206 adsorption of two cellobiohydrolases on microcrystalline cellulose. *Bulletin of*
207 *the Korean Chemical Society*, 2011; 22(7): 716–720.
- 208 14 Camilo M., Fernando G.F., Mariella. R. & Juan. M. G. Hydrolytic enzyme activity
209 enhanced by barium supplementation *AIMS Microbiology*, 2016; 2(4): 402-411.

- 210 15 Zhu Y., Liu G. & Li H. Cloning and characterization of a thermostable
211 carboxylesterase from inshore hot spring thermophile *Geobacillus sp. ZH1*. *Acta*
212 *Oceanol Sin*, 2012; 31(6): 117-126.
- 213 16 Roy S K., Dey S K., Raha S K. & Chakrabarty S L. Purification and properties of an
214 extracellular endoglucanase from *Myceliophthora thermophila* D-14 (ATCC 48104).
215 *Journal of General Microbiology*, 1990;136: 1967-1971.
- 216
- 217 17 Deb P., Talukdar S. A. & Mohsina K Production and partial characterization of
218 extracellular amylase enzyme from *Bacillus amyloliquefaciens* P-001. *Springer Plus*.
219 2013; 2: 1-12.
- 220 18 Speir T. W. & Ross D. J. Hydrolytic enzyme activities to assess soil degradation and
221 recovery, Enzymes in the environment. *Activity, ecology and applications*. Marcel
222 Dekker, New York, 2000; 407- 431.
- 223 19 Li Y. T., Rouland C., Benedetti M., Li F. B., Pando A. & Lavelle P. Microbial
224 biomass, enzyme and mineralization activity in relation to soil organic C, N and P
225 turnover influenced by acid metal stress," *Soil Biology and Biochemistry*, 2009; 41:
226 969977-969975.
- 227 20 Dussault M., Becaert V., François M., Sauvé D. & Deshenes L. Effect of copper on
228 soil functional stability measured by relative soil stability index (RSSI) based on two
229 enzyme activities, *Chemosphere*. 2008; 72: 755-762.
- 230 21 Seregin I.V. & Ivanov V.B. Physiological Aspects of Cadmium and Lead Toxic
231 Effects on Higher Plants. *Russian Journal of Plant Physiology*, 2001; 48: 523-544.
- 232 22 Sharma P, Dubey RS. Lead Toxicity in Plants. *Brazilian Journal of Plant Physiology*,
233 (2005); 17: 1-19.
- 234 23. Hind L. Cellulase of fungal strains from the soil of an extreme environment (soil near
235 thermal springs). Faculty of Natural Sciences and Life. Biotechnology

236 Microbiological engineering science technology and health, Selection of strains and
237 study of enzyme characteristics University of Constantine 1, 127p. 2013. Fench.

238 24 Lee Y. J., Kim B. K., Lee B. H., Jo K. I., Lee N K., Chung C. H., Lee Y. C. & Lee J.
239 W. Purification and characterization of cellulase produced by *Bacillus*
240 *amyloliquefaciens* DL-3 utilizing rice hull. *Bioresource Technology*, 2008; 99(2):
241 378-386.

242 25. Nabti H., Kecha M., Boucherba N. & Benallaoua S. Extraction and purification of a
243 thermostable amylase from a hyperthermophilic archaea Extraction and purification
244 of a thermostable amylase from a hyperthermophilic archaea. *Science et*
245 *Technologie* 2005 ; 23 : 80-85. Fench.

UNDER PEER REVIEW