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Interaction insect-microorganisms producers of ochratoxin A infesting cocoa (*Theobroma cacao*) bean stocks from Tonkpi region, Western in Côte d Ivoire

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ABSTRACT

The storage of cocoa beans in humid tropical area lead to their infection by insects and mold. The objectives of the current study were to make an inventory of insects and molds producing ochratoxin A and to highlight the type of relationship between the incriminate insects and the molds. During the two cocoa campaigns respectively between april and august 2014 and between october 2014 and february 2015, 6 lots of dried beans respectively at each campaign, have been constituted and put in 5 Kg bags. The lots have been analyzed each month during a period of 5 months. The micro-flora has been isolated by the dilution method on Sabouraud agar of chloramphenicol. The developed strains have been identified according to Samson and *al*. classification. The colonies have been enumerated according to the recommended formula by the French Association for Standardization (AFNOR). We found 6 and 3 *Aspergillus* species respectively during the small and great campaign and only a *Penicillium* species during both campaigns. The enumeration after shifting revealed 5 and a single species respectively of Beetles and Lepidoptera at the small campaign and 3 species of Beetles and a single species of Lepidoptera during the great campaign. Two types of relationship were established between insects and molds in cocao bean stocks, the commensalism and the symbiosis. This study revealed more contamination by the insects and molds during the small campaign.

Keywords : *Theobroma cacao*; insects; ochratoxin mold; interactions; Côte d'Ivoire

1. INTRODUCTION

The cocoa (*Theobroma cacao* L.) is the pillar of development many countries. The global crop in producing areas, namely, Latina America, South Asia, and Africa, is estimated to 4.06 millions of tons corresponding to 3 billion of US Dollars [1]. In Côte d'Ivoire, the cocoa speculation constitutes the main pillar of the national economy. With more than 46% of exported products, including more than two thirds of the active population [2]. The ivorian production represents 44% of the world crop with an annual average of 1.700.000 tons [3, 4, 5, 6]. This makes of Côte d'Ivoire the first country cocoa producer. The cocoa farming contributes to more than 10% of Ivorian Gross Domestic Product (GDP) and generates more than 50% for exportation taxes [7]. However, the economic challenge that represents this culture can be compromised, because of necessary of long storage period, which only can guarantee its availability on the market. In fact, the stocks of cocoa beans undergo many damages caused by insects and molds producing ochratoxin A (OTA), a toxic chemical compound. On one hand, this infestation, which could be caused by the metabolism of insects and the secondary metabolism of the two kinds of the microscopic fungus (*Aspergillus* and *Penicillium*) on the other hand, constitute a serious threat for cocoa trade. The OTA causes cancinogenic diseases, immunosuppressive, nephrotoxic and teratogen [8, 9]. Therefore, it is a serious threat for annuity products such as the cocoa. In order to preserve the consumer's health and the producers potentials, the European Union suggests as far as possible the maximum rate of OTA at 2 µg/Kg for cocoa and derivative products [10, 11]. Unfortunately, by fixing such a limit could lead to a loss of an important amount of cocoa. This study was designed to improve cocoa beans storage by determining interactions between insects and microorganisms producing OTA in cocoa beans from Tonkpi region in western part of Côte d'Ivoire.

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2. MATERIAL AND METHODS

2.1. Study site

The cocoa beans samples (bush beans or edge file beans) were taken in Danané (7°15' North Latitude and 8°9' West Longitude) villages' plantation in Tonkpi region, western Côte d'Ivoire. Two sample collections have been carried out, one during the great campaign and the other during the small campaign. The cocoa samples collected were followed and treated in Abidjan port and at the Institut Pasteur de Côte d'Ivoire. During the study period, the abiotic factors, namely, the temperature and the air relative humidity have varied according to the site and the period. In the Tonkpi region, the rainfall varied from 27 to 408 mm. In Abidjan port, from September 2014 to January 2015, the monthly average temperature varied from 27.1°C to 31.62°C. The relative humidity oscillated between 67.7 and 77.10%.

2.2. Identification of insects

The "bush" beans of cocoa came from the pods shelling then dried and were not submitted to any anti-ruiner treatment.

To identify ruiner insects in the stocks of cocoa bean samples have been stored for a month. Then they have been sifted each month during five months. The sieving was done in a muslin cage.The alive or dead adult of insects collected have been observed, identified and counted under a magnifying glasse. The alive insects were put back in corresponding cocoa bean samples. The identification was made based on the identification manuals [12,13]. The insects have counted and recorded separately according to the species. During the storage, the temperature and the relative humidity of the ambient environment have been recorded in order to assess the impact of environmental factors on the infesting potential and the proliferation of the insects' population.

2.3. Isolation and identification producing molds of ochratoxin A in cocoa beans during storage

The "dilution method" or indirect method has been used [14]. Ten (10) grams of the tested cocoa bean samples were put into a 250 ml sterile flask containing 90 ml of buffered peptone water. The mixture is leaved to stand two minutes and then homogenized during five minutes. With a micropipette, 1 ml of the homogenized has been withdrawn and 9 ml of salt tryptone were added. The mixture was shaken for a minute in order to obtain the dilution of 10^{-2}. The later solution was diluted once again with 9 ml of salt tryptone to obtain the dilution of 10^{-3}. 0.1 milliliter of each solution (10^{-2} and 10^{-3}) was radiated on the sabouraud agar containing the chloramphenicol. Three control (W1, W2, and W3) samples were considered (W1, chloramphenicol Sabouraud agar without diluted solutions, W2, chloramphenicol Sabouraud agar including 0.1ml of salt tryptone and W3, chloramphenicol Sabouraud agar containing 0.1ml of buffered peptone water). These control samples and the tests were incubated in a stove at 30°C during 3 up to 7 days. The developed colonies were enumerated according to the recommended formula put forth by AFNOR and expressed in UFG/g.

$$N = \frac{\sum C}{(n1 + 0.1n2)Vd}$$

N: number of colonies per g or per ml of analyzed product.

$\sum C$: Sum of counted colonies at the level of retained boxes of the two dilutions containing a minimum of 15 and a maximum of 300 colonies.

n_1: number of boxes submitted to the dilution of 10^{-2}

n_2: number of boxes submitted to the dilution of 10^{-3}

d: dilution of 10^{-2}

v: volume of the inoculum applied to each box in ml.

The developed strains were observed as humid smears under microscope at a magnification of x10 and x40. The observations were based on the characteristics of the aspergillaire head (presence or absence of metulea), the arrangement and form of phialides, form and height of the vesicle, height, aspect and the color of the conidiophore and conidia.

For the microscopic examination, the texture of colonies (fleecy, cottony, powder, velvety, dusty or granular), their topography (raised plane, cerebriform with radial grooves), the color on the culture reverse as well as the color of the Petri boxes containing the colonies after incubation.

The identification was made according to the classification of Raper and Fennell (1965) [15] and Samson et *al.* (1995) [16].

2.4. Statistical analyses

The statistical analysesis were applied in STATISTICA 6.0 and XLSTAT 7.5 with signicativity level α=0.05.

3. RESULTS

3.1. Idendification of of insects in cocoa beans stored over 150 days stocks

At the 30th day, the inventory revealed only a Beetles order for the big cocoa campaign and Beetles order and Lepidoptera orders at the small cocoa campaigns. At the great campaign, the Beetles order comprised three species namely, *Ahasverus advena* WALTL, *Carpophilus hemipterus* LINNE and *Tribolium casteneum* whereas at the small campaign, four species of Coleoptera were identified (*Ahasverus advena*, *Carpophilus hemipterus*, *Tenebroides mauritanicus* LINNE and *Tribolium castaneum*) and a species of Lepidoptera (*Ephestia cautella*). The predominant species was *Ah. advena* with a proportion of 57.1% and 73.7% respectively at the great and small cocoa campaigns. At the 60th day, the Beetles and the Lepidoptera were found in all the cocoa bean samples. However, during the great campaign, a new species of Lepidoptera order, *E. cautella* was identified in addition to the three Beetles species. The cocoa stocks of the small campaign revealed six species including a new Beetles species, *Araecerus fasciculatus*. The predominant species was always *Ah. advena* with an average number of 5.83±0.75 individuals either 72.9% of the total number. By contrast, during the great campaign, *Tr. castaneum* was become the predominant species with an average number of 4.83±0.98 individuals representing 63.04% of the total number of insects. From the 90th up to 150th day, the Beetles have been the only order identified at the great campaign corresponding to three species, *Ahasverus advena*, *Carpophilus hemipterus* and *Tribolium castaneum*. The predominant specie was *Ah. advena* with proportions of 60.34%, 54.49% and 48.51% respectively at the 90th, the 120th and the 150th day. At the small campaign, at the 90th day, two species of Beetles's order (*Ar. fasciculatus* and *Tenebroïdes mauritanicus*) disappeared from the stocks. *Ah advena* was the predominant species with a proportion of 44.56% of the insects identified. At the 120th day, six species of the Beetles order (*Ahasverus advena*, *Carpophilus hemipetrus*, *Araecerus fasciculatus*, *Tenebroïdes mauritanicus*, and *Tribolium castaneum*) and one species of Lepidoptera order (*E. cautella*) were observed. Among them, it should be noted the reappearance of *Ar. fasciculatus* and *Te. mauritanicus* already reported to the 60th day. The Beetles represented 81.33% of the total number of the insects inventory. At the 150th day, two species of Beetles (*Ar. fasciculatus* and *Te. mauritanicus*) and one species of Lepidoptera (*E. cautella*) disappeared from the stocks. The Beetles order was the only present in the stock with the three species namely, *Ah. advena*, *C. hemipetrus* and *Tribolium castaneum* (Table 1). When we considering sieving period, all listed species were present at 60th and the 120th days in the samples during the small campaign (Tables 1 and 2) while during the great campaign, this was observed only the 60th day.The linear regression models between the number of insects and the relative humidity in the storage room has shown a negative correlation between the two parameters. The number of insects increase with the the temperature. The coefficient equations were as follow: R = -0.94; R = 0.93 for the small campaign and R = -0.571; R = 0.79 for the big campaign. A correlation exists between the number of insects and the duration of cocoa bean storage, R = 0.98 for the small campaign and R= 0.98 for the great campaign.

Table 1 : Average number of insects from cocoa beans from the small campaign for 150 days of storage.

Insect species	Duration storage (days)				
	30	60	90	120	150
Ah. advena	4.66±0.816^{f}	5.83±0.752^{e}	6.83±0.752^{d}	8.667±0.516^{b}	4.83±0.752^{f}
Ar. fasciculatus	0±0^{h}	0.5±0.5477^{h}	0±0^{h}	0.1667±0.408^{h}	0±0^{h}
C. hemipterus	0.66±0.816^{h}	0.83±0.752^{h}	0.33±0.516^{h}	0.5±0.547^{h}	0.5±0.547^{h}
E. cautella	0.33±0.516^{h}	0.5±0.836^{h}	6.5±1.048^{de}	2.33±0.516^{g}	0±0^{h}
Te. mauritanicus	0.5±0.547^{h}	0.1428±0.408^{h}	0±0^{h}	0.1667±0.408^{h}	0±0^{h}
Tr. castaneum	0.1667±0.408^{h}	0.1667±0.408^{h}	1.667±0.816^{g}	7.5±0.547^{c}	15.167±1.169^{a}

ANOVA followed of the test of Stundent-Newmans-keuls at the doorstep of 5%: F=217.314 ; ddl=29 ; P=0.0000

In the same column the averages followed by different letters are are significantly differents. At the day zero, all the values are equivalent to zero.

Table 2 : Average number of insects from cocoa beans from the big campaign for 150 days of storage

Insect species	Duration storage (Days)				
	30	60	90	120	150
Ah. advena	2.33±0.816^{de}	1.67±0.516^{ef}	4.83±0.752^{b}	4.667±0.816^{b}	6.1667±1.169^{a}
C. hemipterus	0.5±0.836^{g}	1±0.632^{f}	0.167±0.408^{gh}	1.33±0.816^{ef}	3.1667±0.752^{cd}
E. cautella	0±0^{h}	0.167±0.408^{gh}	0±0^{h}	0±0^{h}	0±0^{h}
Tr. castaneum	2±0.632^{ef}	4.83±0.983^{b}	3.667±0.818^{c}	5.33±1.032^{ab}	5.5±1.516^{ab}

ANOVA followed of the test of Stundent-Newmans-keuls at the doorstep of 5%: F=50.79; ddl= 19 ; P=0.0000

In the same column the averages followed by different letters are are significantly differents. At the day zero, all the values are equivalent to zero.

3.2. Inventory of the ochratoxigen microflora in cocoa beans

After the drying, the cocoa beans samples contained both genuses of molds: *Penicillium* and *Aspergillus* (Tables 3 and 4). Concerning the genus *Aspergillus*, the cocoa beans of the small campaign were infested with *A. flavus* whereas *A. niger* was found at the great campaign. *Penicillium sp.* was predominant with a contamination level of 88% and 64.8% respectively at the small and great campaigns. After one month of storage, new species appeared, *A. niger* during the small campaign and *A. flavus* and *A. ochraceus* during the great one in addition to those already observed after the drying. The rate of *Penicillium sp.* infestation was still high. For the *Aspergillus* genus, the most represented species were *A. niger* for the small campaign and *A. flavus* for the great campaign. At the second month of storage, this infestation has progressed in the cocoa beans of the small campaign with the appearance of *A. ochraceus*. The number of colonies of this specie was 253.5± 9.092 UFC/g representing 7.38% of the genus *Aspergillus*. *A. flavus* represented 57.33% of *Aspergillus* genus. However, in the cocoa beans of the great campaign, *A. ochraceus* and *A. flavus* listed at the first month of storage have disappeared. At the third month of storage, for the small campaign, three new species have been observed: *A. candidus, Aspergillus sp.* and *A. nidulans*. The cocoa beans were infested by 6 species of *Aspergillus* (*A. flavus*, *A. niger, Aspergillus sp.*, *A. ochraceus*, *A. candidus* and *A. nidulans*) and *Penicillium sp.* genus with a proportion of 90.6% (Table 5). *A. flavus* represented 63.34% of *Aspergillus* genus. At the fourth month of storage, 3 species have disappeared among the 7 which were found in the stock at the small campaign (*A. ochraceus*, *A. candidus* and *Aspergillus sp.*). *A. flavus* was predominant with 6111 UFC/g either 81.79% of *Aspergillus* genus and 77.59% of molds. At the fifth month, *A. ochraceus* and *A. candidus* have reappeared. The samples were contaminated by *Penicillium sp.* (84.85%) and four *Aspergillus* species (*A. ochraceus*, *A. candidus*, *A. flavus* and *A. niger*). *A. flavus* was always the predominant specie of *Aspergillus* genus with 2626.5 UFC/g leading to 81.1% of molds colonies.The principal component analysis revealed that at the small campaign, "*Ah. advena* and *A. flavus*", "*E. cautella* and *A. nidulans*", "*E. cautella* and *A. candidus*" and "*A. niger* and *Te. mauritanitus* " were positively and strongly correlated (Table 5, Figures 1 and 2). However, at the great campaign, "*A. niger* and *C. hemipterus*" were negatively correlated (Table 6, Figures 3 and 4).

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Table 3 : Estimated of colonies the mold ochratoxigenic of cocoa bean stocks in small campaign for 150 days of storage

Duration storage (Days)	Number of colony by mold species (UFC/g)						
	A. flavus	*A. niger*	*A. ochraceus*	*A. candidus*	*A. nidulans*	*Aspergillus sp.*	*Penicillium sp.*
0	303±6.32^{r}	0±0^{w}	0±0^{w}	0±0^{w}	0±0^{w}	0±0^{w}	2411±17.05^{l}
30	607±10.97^{n}	2677.5±194.64^{g}	0±0^{w}	0±0^{w}	0±0^{w}	0±0^{w}	32069.5±143.41^{c}
60	1970.5±30.42^{j}	1213±26.53^{l}	253.5±6.15^{s}	0±0^{w}	0±0^{w}	0±0^{w}	33031±78.98^{b}
90	4545.5±19.77^{f}	977.33±17.42^{m}	253±6.63^{s}	51±2^{v}	103.5±5.64^{u}	152.00±5.01^{t}	69234±55.39^{a}
120	6111±26.84^{e}	1263.00±20.47^{k}	0±0^{w}	0±0^{w}	97.50±1.76^{u}	0±0^{w}	404±98.50^{o}
150	2626.5±38.22^{h}	353.33±8.80^{p}	101.00±6.81^{u}	0±0^{w}	0±0^{w}	153.50±5.85^{t}	18131.5±49.66^{d}

ANOVA followed of the test of Stundent-Newmans-keuls at the doorstep of 5%: %: F= 529722 ; ddl= 41; P=0.0000

In the same column the averages followed by different letters are are significantly differents

Table 4 : Estimated of colonies the mold ochratoxigenic of cocoa bean stocks in big campaign for 150 days of storage

Duration storage (Days)	Number of colony by mold species (UFC/g)			
	A. flavus	*A. niger*	*A. ochraceus*	*Penicillium sp.*
0	0±0^{l}	669.5±22.57^{e}	0±0^{l}	1713±50.07^{c}
30	556.5±9.20^{f}	504.83±10.57^{g}	50.167±0.75^{k}	5376±42.53^{a}
60	0±0^{l}	708±9.87^{d}	0±0^{l}	2475.5±22.71^{b}
90	0±0^{l}	708±19.01^{d}	0±0^{l}	201.167±13.68^{j}
120	0±0^{l}	302.33±12.98^{i}	0±0^{l}	657±7.58^{e}
150	0±0^{l}	202±7.64^{J}	0±0^{l}	451.5±31.29^{h}

ANOVA followed of the test of Stundent-Newmans-keuls at the doorstep of 5%: F=26841,8 ; ddl= 23 ; P=0,0000

In the same column the averages followed by different letters are are significantly differents

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Table 5 : Correlation matrix of variables of the small campaign

	Ah. advena	*Ar. fasciculatus*	*C. hemipterus*	*E. cautella*	*Te. mauritanicus*	*Tr. castaneum*	*A. flavus*	*A. niger*	*A. ochraceus*	*A. candidus*	*A. nidulans*	*Aspergillus sp.*	*Penicillium sp.*
Ah. advena	1	0.318	00582	0.523	0.160	0.276	**0.846**	0.413	0.317	0.284	0.686	0.183	0.293
Ar. fasciculatus	0.318	1	0.637	-0.169	0.047	-0.228	0.091	0.103	0.473	-0.270	-0.116	,426	-0.026
C. hemipterus	0.582	0.637	1	-0.166	0.553	0.058	0.090	0.653	0.327	-0.241	-0.159	-0.151	0.213
E. cautella	0.523	-0.169	-0.166	1	-0.274	-0.144	0.656	0.029	0.502	**0.940**	**0.871**	0.494	0.704
Te. mauritanicus	0.160	0.047	0.553	-0.274	1	-0.331	-0.262	**0.940**	-0.379	-0.340	-0.215	-0.538	-0.005
Tr. castaneum	0.276	-0.228	0.058	-0.144	-0.331	1	0.392	-0.336	-0.132	-0.195	0.049	0.549	-0.301
A. flavus	**0.846**	0.091	0.090	0.656	-0.262	0.392	1	-0.035	0.198	0.400	**0.891**	0.303	0.110
A. niger	0.413	0.103	0.653	0.029	**0.940**	-0.336	-0.035	1	-0.115	-0.055	0.029	-0.348	0.277
A. ochraceus	0.317	0.473	0.327	0.502	-0.379	-0.132	0.198	-0.115	1	0.599	0.181	0.471	0.761
A. candidus	0.284	-0.270	-0.241	**0.940**	-0.340	-0.195	0.400	-0.055	0.599	1	0.660	0.628	**0.836**
A. nidulans	0.686	-0.116	-0.159	**0.871**	-0.215	0.049	**0.891**	0.029	0.181	0.660	1	0.268	0.304
Aspergillus sp.	0.183	-0.426	-0.151	0.494	-0.538	0.549	0.303	-0.348	0.471	0.628	0.268	1	0.539
Penicillium sp.	0.293	-0.026	0.213	0.704	-0.005	-0.301	0.110	0.277	0761	**0.836**	0304	0.539	1

Table 6 : Correlation matrix of variables of the big campaign

	Ah. advena	*C. hemipterus*	*E. cautella*	*Tr. castaneum*	*A. flavus*	*A. niger*	*A. ochraceus*	*Penicillium sp.*
Ah. advena	1	0.681	-0.339	0.767	-0,200	-0.669	-0.200	-0.539
C. hemipterus	0.681	1	-0.012	0.716	-0,222	**-0.829**	-0.222	-0.325
E. cautella	-0.339	-0.012	1	0.287	-0,200	0.428	-0.200	0.167
Tr. castaneum	0.767	0.716	0.287	1	-0,350	-0.524	-0.350	-0.436
A. flavus	-0.200	-0.222	-0.200	-0.350	1	-0.024	**1.000**	**0.897**
A. niger	-0.669	**-0.829**	0.428	-0.524	-0,024	1	-0.024	0.204
A. ochraceus	-0.200	-0.222	-0.200	-0.350	**1,000**	-0.024	1	**0.897**
Penicillium sp.	-0.539	-0.325	0.167	-0.436	**0,897**	0.204	**0.897**	1

In thick, significatives values (off-diagonal) at the doorstept alpha=0,050 (bilateral test)

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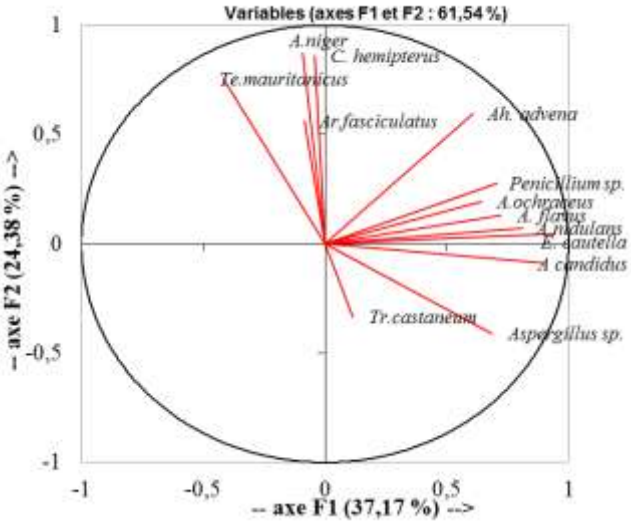

Figure 1 : Circle from the ACP showing the projection of the small campaign in the factorial variables on axes F1 and F2

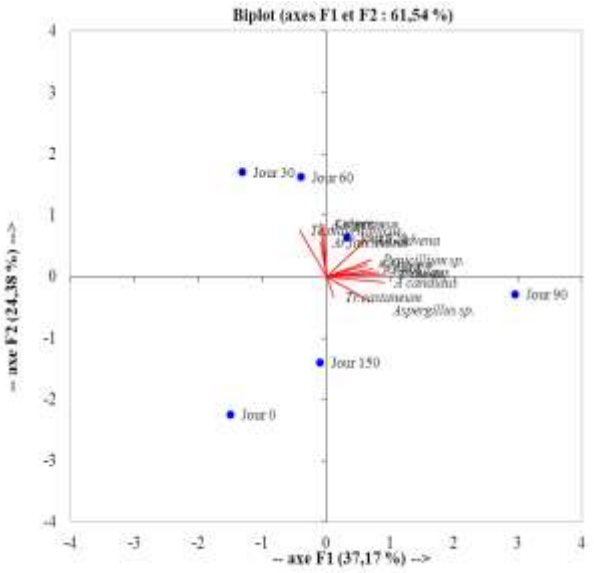

Figure 2 : Factorial correspondences analysis representing to characteristics in Cartesian plan: case of the small campaign

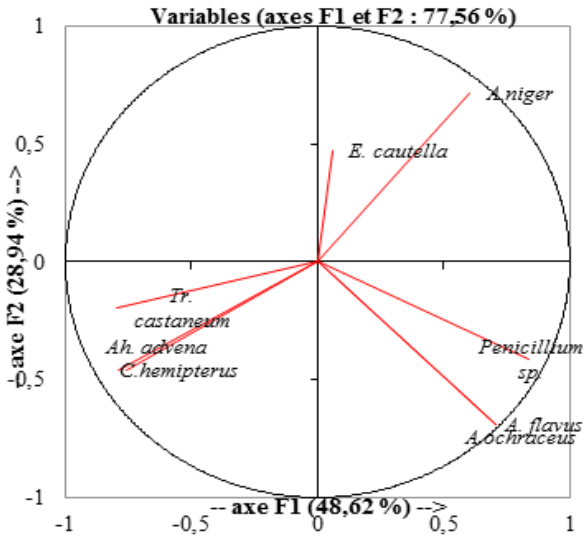

Figure 3 : factorial correspondences analysis representing to characteristics in Cartesian plan: case of the big campaign

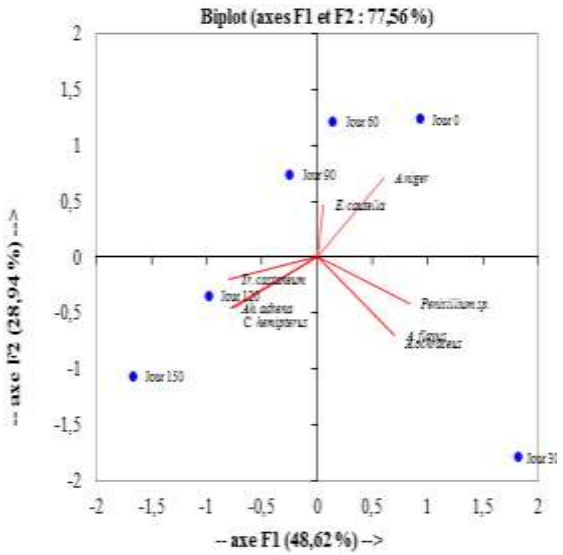

Figure 4 : factorial correspondences analysis representing to characteristics in Cartesian plan : case of the big campaign

4. DISCUSSION

Our study has shown that, in the cocoa beans of the small campaign, six species insects have been listed; while at the great campaign 4 insects' species were present in the stocks. Also, our results revealed that the number of insects was higher in small campaign compared to the great campaign. The inequality in the number of species during both campaigns could be related the variations in abiotic factors notably the temperature and the relative humidity, from one campaign to the other. In fact, the climatic characteristics of cocoa campaign are both different. At the small campaign, corresponding to the big rainy season, the raised humidity promotes the proliferation of insects. The harvested pods have carried some eggs laid by the insects that have colonized the plantation. Hence, the beans have been infested in field. During our works, the presence of some molds in the cocoa stocks during both campaigns after the drying confirms this remark. Our results are similar with those of FAO/WHO/UNEP/ (1999) [17] and Cocoqual (2011) [18] which mentioned that the contamination takes place during the peel removal, the fermentation or the drying. According to these authors, the cocoa beans and the pulp inside the pod being microbiologically sterile, spores have to be introduced by picking material having served to peel removal. In addition, the farmers use the same removal area for different crops. The old pods having not been destroy, decay and were invaded by micro-organisms and insects. The facts that the number of molds colonies and the one of fungal species are different from one campaign to another is also due to the change of climatic conditions. The spores introduced in cocoa beans could be, at first, destroyed the fermentation and also during the drying. Unfortunately, the sampling has taken place in the Tonkpi region where the rainfall is important. To avoid an over-fermentation, farmers planters fermentate their production of the small campaign during only one day. This incomplete process doesn't permit the proliferation of lactic bacterium which appear after 42 to 48 hours of fermentation [19]. That could promote not only the development of present spores in stocks but also new fungal contamination. The short duration of fermentation does not permit the fall of water content, the drying that has followed must reduce the almost totality of water at 7%. But the very short period of sunshine during this campaign, could lead the proliferation of spores and eggs from field on cocoa beans and their propagation in the stocks. These bacteriums secrete some lactid acid, inhibitory substance of molds developpement [18, 20, 21, 22]. Also, the beans peel which constitutes a physical barrier to the infestation lost its compact character. Then this flaky state of peel could promote the massive proliferation of secondary's ravagers insects.

The Works standing on other speculation such as rice and coffee have revealed that the relative humidity and temperatureof rainy season are favorable for molds rising [23, 24]. Moreover, the contamination of cocoa beans during the big campaign could be the fact of using dryings supports previously infested and/ or non-watertight. Our works have revealed that the number of infested fungi augmented with that of insects' ravagers.The raising of fongic species number until the third month of the storage could be the fact of beans' metabolism, molds and mainly the one of insects. In fact, the metabolism of beans, insects produces some heat and humidity. Consequently the elevation of temperature and humidity of cocoa beans stocks could favor molds proliferation. This report agrees with those of Appert (1985) [25] and Lamboni and *al.* (2009) [26] according to whom the heat produce by insects metabolism doesn't evacuating, form some hot spores on the form condensation water promoting fungus proliferation. The presence of *Ah. advena* and *C. hemipterus* seems indicator of the raised humidity rate. In fact, according Delobel and Tran (1993) [13]. *Ah. advena* is characteristic of commodities having undergone an alteration because of a large content of water. Also, these faecal of these insects can serve as development substrate of the molds. The appearance of molds, further to metabolism of insects, beans and their faecal material using as substrate can resemble to a commensalism relation. In addition to their metabolism, insects could also spread fungal spores inside cocoa beans through pores that they create by feeding [27]. These depredators could be considered as some indicators of bad storage conditions, notably the raise humidity rate. The humidity contents superior to 8% promotes molds development in beans [28]. The reduction of fungal species, in the fourth and fifth months of storage, could be related to the existence of competition in the medium for the supply in nutrients, firstly between the molds species themselves and secondly between the molds species and the insects. This argumentation corroborates the one of Lacey (1986) [29] which speculates that some fungal species secrete inhibitory substances of some other growth. Also, to extend their nutritional surface, insects wanted eliminate the mycelial felting, in the same time; consume both this one and the cotyledon reserves. The relations between insects and molds seem to be of benefit to the two protagonists : On the one hand, insects promote molds installation and dissemination in the holes and cracks from the beans perforation caused by the deduction of cotyledon reserves. It could be, here, a commensalism type relation. This commensalism could be explained by the positives correlations between the variables "*Ah. advena* and *A. flavus*", *"E. cautella* and *A. nidulans*", "*E. cautella* and *A. candidus*", "*A. niger* and *Te. mauritanicus*". On the other hand, the mycelia consumed by insects provide to these ones some nutrients necessary to the development and their survival [13]. It could be about a symbiotic relation type. The negative correlation between "*C. hemipterus* and *A. niger*" could be due to this type of relation. In fact, *C. hemipterus* is also micophagous although its presence promotes the proliferation of the *A. niger*, this mold constitutes, of it, a nutritive substrate to remain. The predatory activity of ravagers' insects promotes the beans infestation by the molds.

5. CONCLUSION

The followed of the cocoa beans has shown that infestation is essentially realized by Beetles. Six species of Coleoptera (*Ah. advena C. hemipterus Tr. castaneum, Ar. fasciculatus* and *Te. mauritanicus*) and one species of Lepidoptera (*E. cautella*) have been cropped during our study period. *Ah advena* and *Tr. castaneum* constitute the dominant species of ravagers' insect captured in the samples. The abiotic factors notably the temperature and the relative humidity could exercise an influence on the insects' number, in storage situation.The cocoa beans microbiologic study has revealed that the cocoa stocks were contaminated by *Penicillium sp.* and six species of *Aspergillus* (*A. flavus, A. niger, Aspergillus sp., A. ochraceus, A. candidus, A. nidulans*). The genus *Penicillium sp.* represents the dominant genus of the two cropped genus. As far as the *Aspergillus* genus, the species *A. flavus* and *A. niger* were respectively dominant during the small and big campaign. The contamination level by molds could be results of the number of insects infesting stocks. It exists some correlation between the insects destruction action and the infestation of beans by producing ochtatoxin A from mold in cocoa beans. The relations existing between insects and molds seem to be the commensalism and symbiosis.

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