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Efficacy of Jute Bags Impregnated with Plant Extracts in the Post-Harvest Protection of Cowpeas in Benin

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ABSTRACT

The biological efficacy of jute bags impregnated with *Eucalyptus camaldulensis*, *Cymbopogon citratus* essential oils, and vegetable oil of *Azadirachta indica* was evaluated on the adults of *Callosobruchus maculatus* of cowpea and the molds of the genus Aspergilus and Penicillium, in post-harvest conservation conditions. The results reveal that the jute bags tested have significant insecticidal and antimicrobial activity on *Callosobruchus maculatus* adults, when the dose and exposure time increase from 0 to 2% for 26 weeks.

Introduction

Cowpea (*Vigna unguiculata* L., Walp) is the most important seed legume in tropical Africa (IITA 1982, Zannou 1995 and Diaw 1999). Africa provides more than half of world production with 3.36 million tonnes, 70% of which comes from the West African subregion (Ntare, 1987) with Nigeria, Niger and Burkina Faso as main producers (Jackai and Daoust 1986, Nwokolo and Ilechukwu 1996).

In Benin, despite favorable agro-climatic conditions for cowpea, yields are generally low in traditional cropping systems, between 500 kg and 650 kg / ha (INRAB, 1996). Under favorable conditions, the yield potential can reach 1200 to 1500 kg/ha (Aho, 1988). The losses caused by insects and molds are the most important, because of the tropical and inter-tropical climate very favorable to their development and the type of storage, not very conducive to the fight against insect pests of stocks (Visconti and De girolamo , 2002). In addition, there is a range of technologies available to reduce post-harvest losses, but several constraints persist in the storage or conservation and the processing of cowpea. These constraints are much more accentuated in our markets where cowpea is kept in jute sacks, thus encouraging a very rapid multiplication of stock depredators. Thus, the present study aims to evaluate the efficacy of jute bags impregnated with plant extracts in the post-harvest protection of cowpea.

Material and methods

Equipment

Plant species

Three plant species were used in this study to evaluate their efficacy on cowpea pests in culture. These are *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Azadirachta indica*. The three plants are well known in African pharmacopoeia.

The cowpea

The cowpea seeds used in this study are of the variety "TOLA" and come from the market "Dantokpa" at Cotonou and the market "Ouando" at Porto-Novo, they are packaged in plastic bags, but the sorting of these seeds may be necessary to avoid the use of infected seeds.

Jute bag

Jute bags are purchased in the Dantokpa market. They are then made and dimensioned.

Animal material

The animal material used here is *Callossobruchus maculatus*, a very cosmopolitan species that is thought to be the most damaging to the cowpea stock (Giga and Smith, 1983). For this purpose, two forms are distinguished, namely the sailing or active form and the normal or non-sailing form.

Methods

Impregnation of jute bags

The experimental bags are impregnated with the "solvent-plant extract" mixture and with concentrations of plant extract of: 0%; 0.004%; 0.01%; 0.02%; 0.04%; 0.1%; 0.2%; 0.4%; 1%; and 2%. The 0% concentration solution consists solely of "solvent" and represents the control of the experiment. Once the bags are impregnated with the impregnating solution, they are dewatered and allowed to dry in the shade at room temperature for 48 hours. Twenty (20) bags are thus impregnated to each of concentration.

Method of preparation and screening of cowpea samples

For the preparation and screening of cowpea samples, 500 g of cowpea are introduced into each bag. Infestation of the storage systems is carried out by introducing into each bag 20 pairs of *C. maculatus*. The selection is random. Twenty (20) replicates are made for each concentration. A negative control (free from any treatment) is made, infested

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and replicated in twenty (20) copies. The bags are then stored in areas protected from water. In the warehouse, the bags are labeled and grouped according to treatments.

Population parameters of the insects studied

Stripping is performed every 15 days of storage. During stripping, the following parameters are observed on each bag:

- The number of *C. maculatus* still living;
- The number of dead *C. maculatus*.

The values of these different parameters are collected on sheets previously designed for this purpose.

Microbiological characterization of cowpea during storage

During storage we investigated whether treated cowpea samples are contaminated with the previously identified mold types. It consists in sowing the seeds of treated cowpea on Sabouraud medium in petri dishes and then incubated at 25° C. for 5 days. The first reading is made from the third day. At the end of the five days of culture, the various mold strains are isolated one by one on culture medium such as MEA, DRBC, Sabouraud and PDA for further identification tests.

Determination of the conservation date

The shelf life is determined by the duration of the insecticidal/repellent effect of the plant extracts studied. Indeed we have determined the duration of each plant extract to lose its insecticidal power.

Results and discussions

Results

Making and impregnation of jute bags

The made jute bags are then soaked with the plant extract, drained and allowed to dry in the shade at room temperature for 48 hours as shown in Figure 1 below.

Figure 1: Jute bags impregnated with plant extracts

Cowpea conservation

Figure 2: Cowpea conservation.

Variation of the number of live insects as a function of time

Table I. Effect of plant extracts, application rates and time on the storage life of cowpea bags: result of the regression of fish.

Sources of variation	Chi2	Pr (>Chi2)
Plant extracts	70658	0,000
Doses	1701	0,000
Time	2864	0,000
Plant extracts × Doses	657	0,000
Plant extracts × Time	2953	0,000
Doses × Time	5295	0,000
Plant extracts × Doses × Time	639	0,000

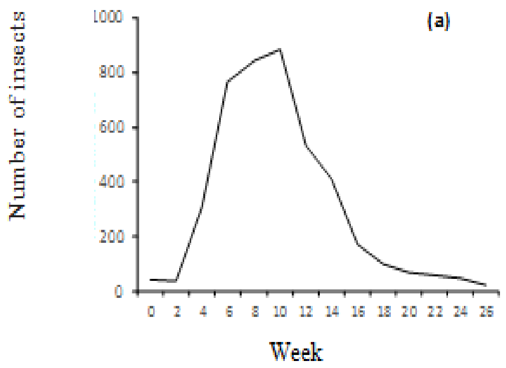
In Table I were presented the results of the generalized linear regression, family of fish carried out on the shelf life of bags of cowpea impregnated with the different plant extracts and according to the doses applied. It appears that the main factors as well as their interactions induced significant differences (P <0.05) in the maximum shelf life of cowpea bags, which reflects a variation in the shelf life according to the extract of plant used, but also of the dose and time (and vice versa).

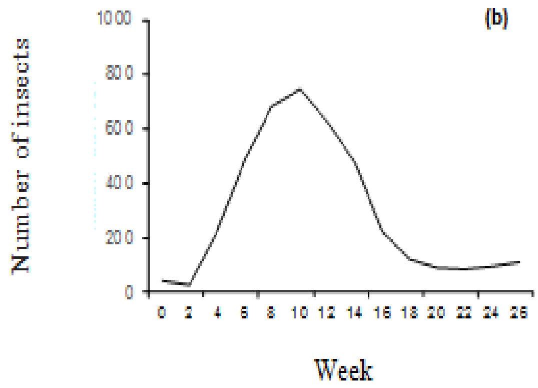
Table II shows the number of living insects in the cowpea stocks according to the doses of the various plant extracts used. Overall, regardless of the plant extract considered, an increase in the dose of plant extracts induced a decrease in the number of insects.

Table II. Average number of insects as a function of the doses of the various plant extracts used (average: Avg, standard deviation: SD).

Doses (µl)	*A. indica*	*C. citratus*	*E. camaldulensis*
	Moy ±SD	Moy ± SD	Moy ± SD
0,4	316 ± 323	12 ± 15	5 ± 11
1	311 ± 321	9 ± 13	4 ± 11
2	308 ± 321	7 ± 13	4 ± 11
4	303 ± 322	4 ± 11	3 ± 11
10	295 ± 318	3 ± 11	3 ± 11
20	288 ± 315	3 ± 11	3 ± 11
40	250 ± 282	3 ± 11	3 ± 11
100	367 ± 316	3 ± 11	3 ± 11
200	144 ± 257	3 ± 11	3 ±11

The variation in the number of insects in time according to the plant extracts used has been presented in Figure 3. It emerges that for both the control (Figure 3.a) and for the extract of *A. indica* (Figure 3.b), the number of insects increased exponentially until reaching the peak at the tenth week (950 insects for the control and 750 for *A. indica*). From the eleventh week onwards, this number decreased considerably to zero for the control and stabilized at 50 insects for *A. indica*. In addition, with cowpea treated with the essential oil of *C. citratus* (Figure 3.c) and *E. camaldulensis* (Figure 3.d), the number of insects in the stocks was zero from the eighth week for *C. citratus* and the twelfth week for *E. camaldulensis*.





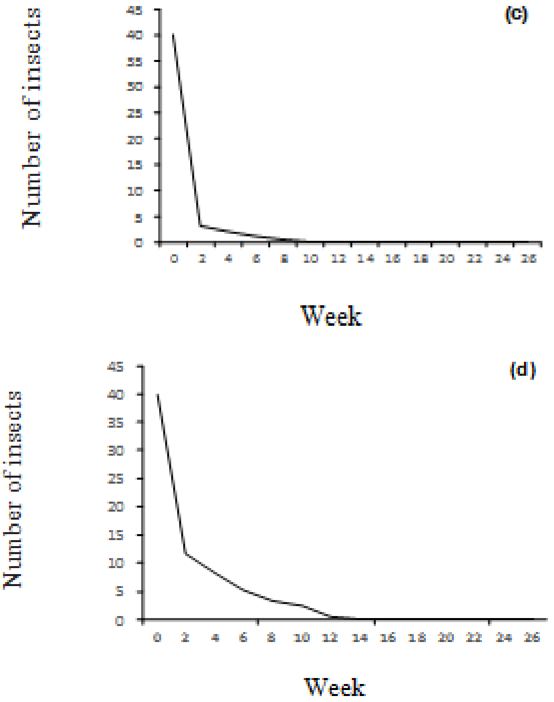


Figure 3. Variation in the number of insects of different oils as a function of time.

Fungal characteristic of cowpea after post-harvest conservation

In Table III were presented the results of the contamination rate of cowpea preserved or not by fungi. The analysis of this table shows that after twenty-six weeks of preservation, no mold was observed in the samples treated with essential oils, which was not the case for the control and the samples treated with the vegetable oil of *A. indica*.

Table III. Contamination rate of cowpea preserved or not by fungi.

Contamination	Aspergillus	Pénicillium
witness	70%	30%
E. camaldulensis	0%	0%
C. citratus	0%	0%
A. indica	65%	35%

Shelf Life Limit

Table IV shows the results of the shelf life limit. The analysis in this table shows that the shelf life limit varied according to the plant extract and the dose.

Table IV. Shelf Life Limit versus Dose (%) of Treated Cowpea Samples.

	Doses	*E.camaldulensis*	*C.citratus*	A.indica	witness
Time limit of retention (Weeks)	0,004	26	26	2	2
	0,01	26	26	2	2
	0,02	26	26	2	2
	0,04	26	26	2	2
	0,1	>26	>26	2	2
	0,2	> 26	> 26	2	2
	0,4	> 26	> 26	2	2
	1	> 26	> 26	2	2
	2	> 26	> 26	14	2

Discussion

The efficacy of essential oils and vegetable oil of *A. indica* is observed over a period of 26 weeks or more than 6 months from the date of impregnation treated bags containing cowpea. The results obtained following the infestation with *C. maculatus* of cowpea contained in jute sacks impregnated with 0.004%; 0.01%; 0, 02%; 0.04%; 0.04%; 0.1%; 0.2%; 0.4%; 1% and 2% reveal a remarkable level of protection when bag treatments are 0.04%, 0.1% and 2% respectively for *E. camaldulenis*, *C. citratus* and *A. indica*.

Indeed, after 4 weeks of storage at a dose of 0.04%, the level of inhibition of insect attack is 100% for the essential oil of *E. camaldulenis*. Similarly, after 2 weeks of storage at a dose of 0.1%, the level of inhibition of insect attack is 100% for the essential oil of *C. citratus*. By cons, after 10 weeks of storage at a dose of 2%, the level of inhibition of attack of insects is 100% for the vegetable oil of *A. indica*. Throughout this experiment, no insect was found in the bags of cowpea treated with essential oils at a dose of 0.1%. It appears that the essential oils of *E. camaldulenis* and *C. citratus* in addition to inhibiting the proliferation and attack of cowpea stocks by *C. maculatus* also has a repellent capacity thus preventing the entry of other populations of pests in the bags. In 2008, Ndomo *and al*. established the repellency of the essential oil of callistemon viminalis against adults of *A. obtectus*. Similarly, Tchoumbougnang *and al*. (2009) confirm its larvicidal effect in insects such as mosquitoes. Earlier, Ketoh *and al*. (1998) said that the essential oil of *Cymbopogon citratus* alters the eggs of the beetle *Callosobruchus maculatus*. Although there is a relative reduction in the density of *Callosobruchus maculatus* populations following the doses of essential oils of *E. camaldulenis* and *C. citratus* introduced into the jute bags. It is important to indicate that the highest concentration (2%) tested in this study does not completely inhibit the development of *Callosobruchus maculatus* in *A. indica* vegetable oil cowpeas bags within 6 months storage.

The reduction of the living insect population in the treated bags is also related to the reduction of egg-laying in adults exposed to essential oils as revealed in numerous studies conducted by Koumaglo *and al*. (1996); Glitho et al. (1997) and Seri-Kouassi *and al*. (2004). In general, the level of inhibition of *C. maculatus* is proportional to the concentration of *A. indica* essential oil or vegetable oil in jute bags. This level, on the other hand, is inversely proportional to the storage time of the impregnated jute bags. The microbiological analyzes carried out on the treated cowpea seeds show that only the vegetable oil of *A. indica* could not completely reduce the fungal flora during storage. This implies that all mold strains do not resist essential oils during storage. The work of De Billerck (2001) shows that the terpene phenols of essential oils against fungi cause several damage such as morphological disturbances of mycelial hyphae, rupture of the plasma membrane and alteration of the mitochondrial structure. This explains the total disappearance of mold after 26 weeks of storage. These results therefore show the effectiveness of the essential oils used in the conservation of cowpea in Benin. This efficacy is due to the presence in these essential oils of bioactive molecules such as terpenoids. These antimicrobial agents destroy the plasma membrane irreversibly, leading to cell death by a lytic process (Pfohl-Leszkowicz, 1999). This property facilitates their insertion between the membrane phospholipids and ensures their solubilization in the lipid bilayer. This results in a destabilization of the structure of the plasma membrane and a change in its permeability to ions, protons and other cellular constituents (Yayi-Ladekan *and al.*, 2011). In addition to induced membrane alterations, these molecules can cross the lipid bilayer, penetrate inside cells and interact with intracytoplasmic targets (Adjou and Soumanou, 2013). In addition, the terpenic phenols of the oils cause fungi, several damage such as morphological disturbances of mycelial hyphae, rupture of the plasma membrane and alteration of the structure of mitochondria (Adjou and Soumanou, 2013). The retention period is 14 weeks (3 months) at 2% for *A. indica*, 26 weeks (more than 6 months) for *C. citratus* and *E. camaldulensis*, whatever the dose applied.

Conclusion

In general, the number of live insects and the rate of microbial contamination are proportional to the concentration of *A. indica* essential oil or vegetable oil in jute bags. These results also show that the plant extracts used have a good insecticidal and antimicrobial action against cowpea pests according to the biological parameter targeted. It is therefore very interesting to continue this work in a dimension of industrializing the results of its work which will not only guarantee the food security of the populations by a better conservation of the cowpea, but it will also open a track for the valorization of aromatic plants from which the essential oils can be extracted for commercial purposes thus increasing farmers' incomes.

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