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Effects of MITC Released from *Boscia Senegalensis* as Biopesticide in Senegalese Seeds with Special Attention to Cowpea: Detection of Residues

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Abstract

B. senegalensis leaves and fruits are known to contain glucocapparin which hydrolysis by endogenous myrosinases lead to the release of methylisothiocyanate (MITC). This product is very active against insect stored products by fumigation. This paper reveals that MITC penetrate into seeds during treatment. Multiple Headspace Extraction coupled to GC (MHE-GC) method allowed to evaluate the level of MITC residues sequestrated by exposed seeds to *B. senegalensis* organs. Furthermore, tests conducted on germination capacity have been demonstrated that MITC doesn't corrupt the germination capacity of grains.

Keywords: *Boscia senegalensis* – MITC- seeds – MHE – residue - germination

1. Introduction

Control of pests of stored product in Africa remains a challenge. The developed countries have proven techniques to reduce pest damages and losses to economically sustainable levels while the developing countries particularly western Africa still experience high post-harvest losses. Labeyrie (1982) reported that African farmers work for insects and the situation has not improved since. It is very uncommon to find recent studies on losses conducted in warehouses or farmers' granaries during storage, but it is generally accepted that losses due to insect pests can exceed 30% (Ratnadass & Sauphanor, 1989; Delobel & Tran, 1993). Our observations show that Tribolium castaneum (Herbst) causes less loss to whole grain (Guèye and Delobel, 1999) since it prefers mostly the germs. Even the occurrence of a natural enemy of *Prostephanus truncatus* (Horn), *Teretrius nigrescens* (Lewis) in farmers' storage in Benin doesn't hamper damages caused by this pest, which stands at 44% in six months (Meikle et al., 2002). Laboratory evaluation showed that when maize was unprotected, damages and losses due to *Sitophilus zeamais* (Motschulsky) reached 20 and 40% respectively in 4 and 8 months of storage (Guèye et al., 2012). For countries seeking to achieve food security in the light of climatic change (floods and droughts) and lack of storage infrastructure, these losses are major challenges in achieving the food security objective.

Products such as cowpea and groundnut remain important staple foods and cash crops. These have always suffered high pest damages by *Caryedon serratus* (Olivier) and *Callosobruchus maculatus* (Fabricius) during storage and can cause 80 to 100% losses within few months (Gomez, 2004; Amevoin et al., 2007). Groundnut is of high economic importance in Senegal where warehouse storage (called seccos) serves as a central storage. The control of insect pest of stored products (mainly C. serratus) was almost exclusively based on the use of fumigants (methyl bromide and phosphine) and insecticide dust. These grains were used often as seeds during the subsequent rainy season. However, at the individual level, farmers used any means available in the control of pests. Before the advent of pesticides, insecticidal plants and to a lesser extent inert dusts like ashes and sand were the main materials used for insect pest control. The success of pesticides during the three last decades has somewhat relegated the use of local materials. Lack of basic training, insufficient financial resources of farmers and numerous cases of poisoning and environmental hazards has drawn attention safety means of control pest (Guèye et al., 2011). Indeed, insecticidal plants in the local biodiversity are alternatives to synthetic chemical insecticides.

Many studies have been conducted on the basis of use of plants or extracts. High susceptibility of cowpea to bruchid vis-a-vis the role of cowpea in ensuring food security among rural populations has led to many of studies on this legume (Seck et al., 1993; Sanon et al., 2005; Doumma and Alzouma, 2008). *Boscia senegalensis*, a common plant from Senegal to the horn of Africa is very abundant in the Sahelian zone and has been widely used in this context (Baumer, 1995). Its efficacy due to glucocapparin hydrolysis by endogenous myrosinases (thioglucoside glucohydrolase, EC.3.2.3.1) which lead to the release of methylisothiocyanate (MITC) has been largely proven (Seck, 1994; Morra & Borek, 2010). A study just carried out (Guèye et al., 2013) showed significant differences in glucocapparin level in leaves and fruits of B. senegalensis harvested in four localities of Senegal during two years. Additionally, it was revealed that during rainy season, plant parts showed lowest glucocapparin content but the maximum occurred two months later, in January. Previous studies have not investigated the occurrence of residues that may result from application of *B. senegalensis* to stored seeds. MITC is known to have insecticidal, nematicidal and fungicidal properties (Wales, 2002). When liberated from pesticide precursors as metam sodium and dazomet (Zheng et al., 2006), it is also considered as an atmospheric, aqueous and soil contaminant and has a respiratory and eye irritant actions (Dourson et al., 2010; Swancutt et al., 2010).

The aim of this work is to evaluate occurrence of MITC in cowpea and other cereal and legume seeds treated with B. senegalensis. We also detect the ability of seeds to germinate after exposure of increasing doses of MITC derivated from *B. senegalensis* crushed leaves and fruits.

2. Materials and Methods

2.1 Plant Material

2.1.1 The Substrates

These were cowpea (*Vigna unguiculata* L. (Walp)), maize (*Zea mays* L.), millet (*Pennisetum typhoides* L.) and peanut (*Arachis hypogaea* Ol.). Seeds were sieved to remove immature seeds and foreign material. To eliminate hidden infestation, seeds were kept in the freezer for a week before use.

2.1.2 Boscia Senegalensis

Fruits and green leaves of *B. senegalensis* were harvested at Dakar. Harvest was always done early in the morning and plants immediately tested on insects. Germination tests and determination of quantities of MITC were carried out on the same batches of biological material (seeds).

material (seeds).

2.2 *Experimental Procedure*

2.2.1 Germination Tests

For each dose, germination tests were performed at the end of the treatment. Three controls were treated under the same conditions. 100 seeds were randomly placed on water-soaked cotton spread in a Petri dish of 12 cm diameter. The experiment was replicated 3 times and germination rate calculated after 10 days. Other trials were conducted with cowpea which had been stored for two years with the treatment of B. senegalensis leaves at the rate 2g of leaves/100g of cowpea. Results were subjected to ANOVA using the Minitab 16 Statistical Software. Differences between treatments were determined by multiple comparisons using the Tukey test at $P < 0.05$.

2.2.2 Analytical Standards

A 4-point calibration curve was constructed for MITC under MHE conditions (see below). MITC (97 %) was purchased from Sigma Aldrich (Bornem, Belgium). MITC spike solutions were prepared in diethyl ether at 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml, respectively. Actual analytical standards were obtained by spiking 100 µL of the respective MITC spike solution onto a small piece of filter, which was contained in a 20 mL headspace vial containing 1 g of untreated seeds (with screw cap and PTFE lined septum). In order to assist ether evacuation, the vial was kept open to air for 2 min. Final concentration levels on filter were 1 µg, 10 µg, 100 µg and 1000 µg. Toluene-D8 was used as reference standard to normalize MITC peak area prior to MHE data processing.

2.2.3 Quantification of Residues

Residual MITC in both leaves and fruits treated grains was determined using multiple headspace extraction (MHE) in combination with GC/MS in full scan and SIM mode. Plants were treated with both fruits and leaves of *B. senegalensis*. Approximately 4 g of either fruits or leaves were mixed with 100g plant material. Final amounts were between 2 and 2.5 g. Residues concentrations are expressed in mg residual MITC per g.

A Thermo Scientific Trace 1300 GC instrument (Interscience, Louvain-la-Neuve, Belgium), equipped with Thermo Triplus RSH autosampler and i-connect S/SL injector module, was hyphenated to a Thermo ISQ single quadrupole MS. The autosampler was fitted with MHE vent tool. Separation was achieved using a 20 m × 0.18 mm I.D., 1 µm df Rxi-624 Sil MS capillary column (Restek, Bellefonte, PA, USA). Experimental details are summarized in Table 1.

3. Results

3.1 Evaluation of Germination Rate of Boscia Senegalensis Treated Seeds

The experiment showed that such treatment using crushed *B. senegalensis* leaves and fruits over ten days does not have a significant effect in exposed seeds to MITC liberated from glucocapparin ($P < 0.05$). High levels of germination were recorded with millet, cowpea, groundnut and maize regardless concentration (table 2). Another experiment with cowpea kept during 24 months gave a germination rate of 84±6%.

3.2 Quantification of Residual MITC in Cowpea

Table 3 shows parameters of linear regression derived from cowpea treated with *B. senegalensis* fruits and leaves and total amount of MITC found in their two organs. Concentrations of MITC were four times high in fruits (2.03mg/g) than in leaves (0.55 mg/g).

Figure 1 and 2 described plot of data and linear regression fit of *B. senegalensis* fruits and leaves, respectively. Based on triple replications, fruits and leaves has the same $R^2 = 0.9865$.

Figure 3 depicts the full scan chromatogram. Insert shows the ion trace extracted at m/z= 73, which is typical for MITC. A least, three other main peaks were also identified: unknown molecule at 2.33 min, Ethyl acetate (3.91 min) and Hexenal (5.98 min).

4. Discussion

The results presented herein showed the release of MITC by *B. senegalensis* fruits and leaves similar to the observation of Seck (1994). However, concerning sequestration of MITC by seeds subjected to *B. senegalensis*, information is limited. For the past two decades that *B. senegalensis* had been studied, only Sanon's *et al.* (2005) had conducted studies related to MITC liberated by *B. senegalensis*. Judging from the difference between the amount of MITC introduced into jar and the remaining after the experiment, they suggested that MITC was absorbed by cowpea seeds. In our study, natural MITC directly produced by *B. senegalensis* raw material was measured after 10 days exposure using the MHE method. It has been proved that all seeds tested namely, millet, maize, cowpea and groundnuts have sequestered MITC. Using a simple method, comprising aeration of seeds treated for 24 hours at 30°C to liberate the MITC molecule, still revealed significant amounts of MITC in all types of media. This is due to the fact that boiling point of MITC is high (118°C). Thus, it appeared important to establish kinetic desorption of MITC over time in all these kinds of seeds. This is because composition and texture are different for different seed types; for example high seed oil content would have greater influence on MITC liberation.

As a result, larvae of stored grain products such as *P. truncatus*, *C. maculatus*, *C. serratus* and *S. zeamais* can be controlled by MITC. Nevertheless, the appropriate level of sequestration and time of exposure should be determined for efficiency in the control. Indeed, an ongoing study being undertaken to determine the efficacy of *B. senegalensis* against major insect pests of stored products of legumes and cereals revealed that efficient doses required to kill adults will require twofold or forthfold increases, depending on insect species, (Guèye *et al.*, Unpublished results).

Tests conducted on germination demonstrated that MITC doesn't disrupt the germination capacity of seeds. No significant statistical difference was found between control and treated seeds with doses of *B. senegalensis*. Furthermore with cowpea seeds treated with *B. senegalensis*, leaves kept in an incubator at 30°C during 24 months, had the same level of germination as the control (84±6%). On the basis of germination rate, Kéita *et al.* (2002) did not find significant effect with seeds treated with aromatized powder of *Ocimum basilicum* and *O. gratissimun* after 5 days exposure (88%). Indeed, low level of emergence rate is a major concern of smallholder farmers in Senegal largely due to insect pest attacks during storage.

According to the MHE investigation, further studies are necessary to determine the level of MITC residues in food prepared from *B. senegalensis* (fruits and leaves) protected grains. It is also strongly recommended to measure MITC in all *B. senegalensis* based edible products before use as food. Currently in Niger, several products from *B. senegalensis* are being developed for human consumption with intensive promotion going on. In this context, the measurement of residual MITC is essential for determining food safety status. The MHE method reported in this paper could be systematically carried out.

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Table 1. Overview of experimental settings

Parameters	**Settings**
MHE method	
Incubation time	5.00 min
Incubation temperature	150°C
Injection volume	1mL
# Extractions	3
GC method	
GC oven	35°C (5.00 min) → 175°C (0.00 min) @ 20°C/min
S/SL temperature	240°C
Split flow	15 mL / min

Carrier gas	Helium @ 1.5mL/min
MS method	
Mode	Full scan
Mass range	29 – 400 amu
Scan time	0.2 sec

Table 2. Germination of seed treated with *Boscia senegalensis* fruits (Fr) and leaves (L). *Tests showed any difference between controls and treated seeds (n = 300; P<0.05).*

Doses (g/l)		Peanut	Niebe	Maize	millet
Controls		87±2	96±3	87±3	83±3
0.5	Fr	92±3	98±0	87±11	80±2
	L	91±4	96±3	91±1	84±4
1	Fr	87±1	94±5	87±11	81±1
	L	88±5	94±4	82±15	84±4
2	Fr	85±4	96±2	88±7	82±3
	L	83±4	97±3	78±11	87±3
3	Fr	85±3	97±1	87±6	77±1
	L	86±8	95±4	92±2	83±4
4	Fr	86±6	96±1	87±5	88±2
	L	87±4	98±1	88±1	84±2

Table 3. Results from linear regression of *Boscia senegalensis* fruits and leaves and quantities of MITC

	Fruits	**Leaves**
Area		
0	4683253	1183152
1	4542755	1147657
2	4451900	1124704
F	73.197	73.197
df	1	1
MITC (mg/g)	2.03	0.55

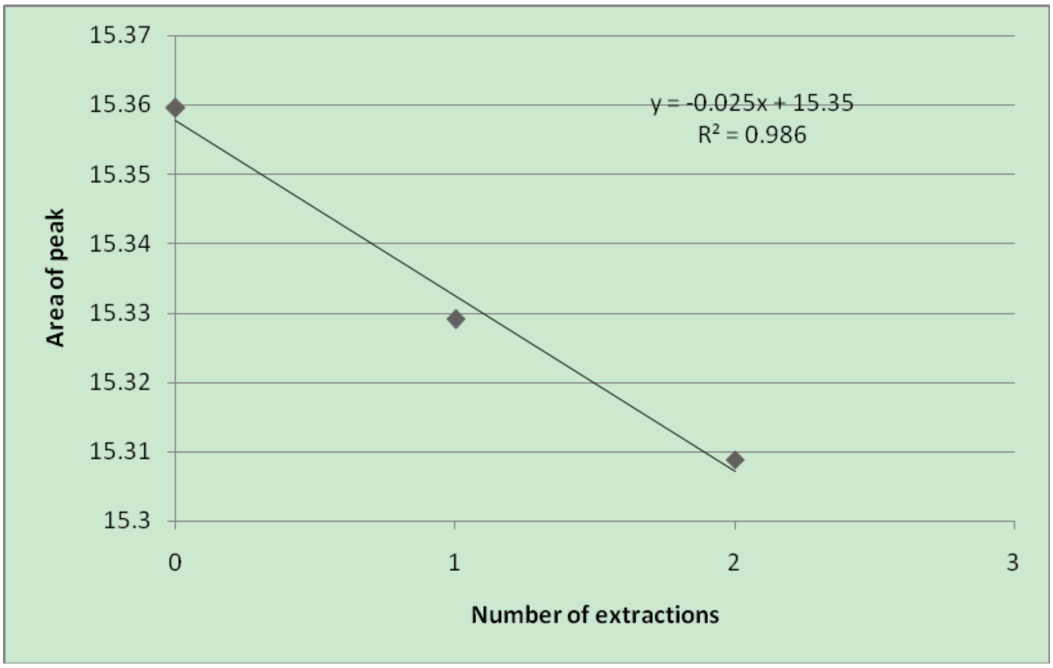

Figure 1. Plot of data and of linear regression fit for MITC from Boscia senegalensis fruits by MHE

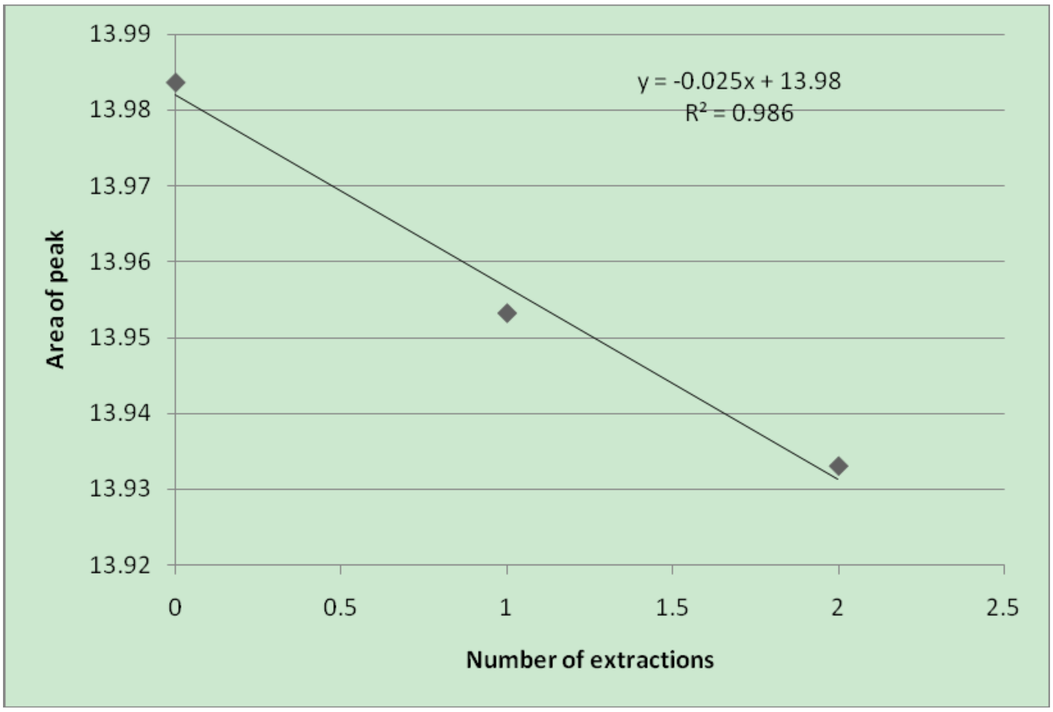

Figure 2. Plot of data and of linear regression fit for MITC of *Boscia senegalensis* leaves by MHE

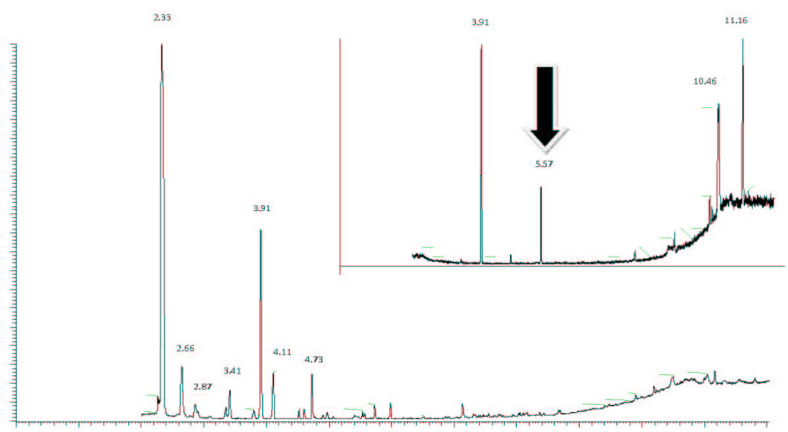

Figure 3. Chromatogramm of cowpea with MITC (arrow with the insert – RIC on m/z= 73)