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Ocimum gratissimum essential oil and modified montmorillonite clay, a means of controlling insect pests in stored products

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ABSTRACT

The insecticidal properties of formulations based on *Ocimum gratissimum* essential oil and montmorillonite clay have been improved after modifications of the clay. Insecticidal tests have been conducted against the maize weevil *Sitophilus zeamais*. The mortality of *S. zeamais* decreased from 100% to 95%, 87% and 0% after 7 days, respectively, for the essential oil adsorbed on modified clay, unmodified clay or used without adsorbent. The formulation prepared with unmodified clay completely lost insecticidal activity after 30 days, whereas the formulation with modified clay lost about 60% of its full insecticidal potency in the same time. The insecticidal effects of the essential oil persisted for about 7, 45 and 80 days respectively for crude essential oil; after adsorption on unmodified and after adsorption on modified clay. The findings suggest that formulations based on essential oils adsorbed on modified clays can be considered as alternatives to synthetic insecticides for use in stored product protection.

1. Introduction

The global post-harvest grain losses caused by insect damage and other bio-agents range from 10 to 40% (Papachristos and Stamopoulos, 2002). Small-scale farmers may lose as much as 80% of their stock due to insects after storing for 6–8 months (Kitch et al., 1992; Nukenine et al., 2002). In the northern provinces of Cameroon, where cereals play an essential role as human food, the maize weevil *Sitophilus zeamais* Motschulsky (Curculionidae) is the main pest in granaries (Ngamo Tinkeu, 2004). Damage due to insects affects mainly the quality, quantity, commercial and agronomic values of the product (Bell et al., 1998). Although synthetic insecticides are commonly used to reduce these losses, there is global concern about their negative effects such as environmental pollution, pest resistance and pesticide residues in food (Ogendo et al., 2008). The continuous application and excessive reliance on chemical pesticides have also resulted in toxicity hazards for non-target organisms and users (Isman, 2006). In recent years, studies have been focussed on the use of plant essential oils and their bioactive chemical constituents as possible alternatives to synthetic insecticides (Rajendran and Srianjini, 2008). Research has shown that plant essential oils have a potential use as fumigants; they are locally available, and have low toxicity and rapid degradation (Sha Sha et al., 2010).

Although many aromatic plants have recently been proven to have insecticidal effects, the active components are not persistent. Essential oils of *Ocimum gratissimum* have been tested as grain protectors in Cameroon but due to their high volatility, their insecticidal effect tends to be transient (Ngamo Tinkeu et al., 2007). The challenge is now to develop a formulation that can remain active against insects for a long period. The clay, which is a common constituent of soils and sediments, is used in numerous formulations of insecticides, drugs and cosmetic powders. In crop protection, the clay alone can be used to help control pest infestation as an inert dust (Keita et al., 2001). Insecticidal activity of clay and *Xylopia aethiopica* essential oil formulations has already been investigated; and shown to increase the persistence of the toxicity and stability of the essential oil (Nguemtchouin et al., 2010). This period of persistence is however insufficient for the protection of stored products. To avoid continuous application and repeated treatment, which could result in development of target pest resistance, the present study aimed to improve the stability of the insecticidal effect of powder formulations.

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2. Materials and methods

2.1. Essential oil distillation

The *O. gratissimum* plants used as source of essential oil were collected from the Ngaoundere Adamaoua region (Cameroon) in July 2010. Fresh whole plant samples were placed in labelled bags and transported to the laboratory. Fresh leaves of plant were hydrodistilled in a modified Clevenger-type apparatus for 4 h, extracted and then dried over anhydrous sodium sulphate. The essential oil was then stored in airtight containers in a refrigerator at 4 °C until its use.

2.2. Essential oil analysis

Quantitative and qualitative analysis of *O. gratissimum* essential oil were performed using gas chromatography and mass spectroscopy. Concerning quantitative analysis, essential oil was analysed by a 14B Shimadzu Co. apparatus fitted with a flame ionisation detector, an HP-3395B integrator and a fused silica capillary column [30 m × 0.25 mm ID, 0.25 μm film thickness coated with a 5% phenyl 95% dimethylsiloxane stationary phase (HP-5MS from Agilent) at 230 °C]. The detector temperature was 250 °C. Nitrogen was used as carrier gas at 1.5 mL min^{-1}, hydrogen at 30 mL min^{-1} and air at 250 mL min^{-1} (FID detector). The column temperature programme was: an initial temperature of 40 °C (hold 5 min), a ramp at 5 °C min^{-1} from 40 °C to 200 °C and at 10 °C min^{-1} from 200 °C to 230 °C with a final hold at 230 °C for 10 min. The sample (1 μL) was injected with a split ratio of 1:10. The identification of essential oil components was undertaken firstly by comparing their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from the literature after analysis with GC–MS in the same conditions as used in GC–FID analyses, and. secondly comparing their retention indices with those in the literature or with those of authentic compounds available in the laboratory. The retention indices were determined in relation to a homologous series of n-alkanes (C_8–C_{24}) under the same operating conditions.

2.3. Insect rearing

The used maize weevil *S. zeamais* was of the strain 01Z/LN/01 *in vivo* collection of insect grain pests of Storeprotect Laboratories at the University of Ngaoundere. This strain has been in collection since 2008 in an incubator monitored at local temperature (28 ± 4 °C). Adult insects used for tests were three weeks old.

2.4. Preparation of clay powder

A local montmorillonite was collected in Maroua locality in the far North of Cameroon. After collection, stones and other heavy particles were manually removed from the sample, which was then kept dispersed in ultra pure water for several hours. Fractions less than 50 μm were obtained by using an appropriate sieve. This specific small particle size was used to improve the adsorption capacity of our clay material. Prior to use, clay was firstly converted to montmorillonite-Na^+ form (Mont-Na) to prepare for treatment with cethyl trimethyl ammonium. The preparation of Mont-Na was performed by dispersing raw montmorillonite (<50 μm) in a sodium chloride solution (1 mol L^{-1}) over 24 h to replace all exchangeable cation content in the clay by Na^+. The product was then washed with deionised water until free of chloride as indicated by the $AgNO_3$ test. The Na-clay was dried at 70 °C and ground to pass through a 50 mesh sieve. The treated Na-clay was designated Mont-Na.

2.5. Preparation of modified clay

All chemicals of analytical grade and water were obtained from a Milli-Q system (resistivity 18.2 Ωm). For organic modifications, fresh solutions of organic solutions were obtained by solution of an appropriate quantity of cethyltrimethylammonium chloride (CTMAC) 25% in ultra pure water. Organic solutions prepared at 0.1 mol L^{-1} were immediately used for clay modification.

Organo-clays were synthesized according to procedure described by Bouras, Unaobonah, Reddy and co-workers (Bouras et al., 2007; Unuabonah et al., 2008; Reddy et al., 2009). A given mass of montmorillonite-Na previously obtained was dispersed in ultra pure water in a proportion of 0.5% (W/W) and 500 mL of CTMA solution was added (8 mL min^{-1} using a peristaltic pump) to the suspension previously stirred for 1 h. The resulting suspension was aged for 24 h at room temperature. After reaction, the clay sample was separated by centrifugation and washed repeatedly with ultra pure water. The washing and centrifugation steps were repeated until complete removal of the foam formed due to the surfactant. The resulting clay sample was dried at 40 °C for 24 h, ground in an agate mortar to a fine powder and identified as Mont-CTMA and fractions of Mont-CTMA less than 50 μm were obtained by passing the final modified sample through a sieve of 50 μm mesh.

2.6. Bioassay procedures with crude essential oil

Bioassays were conducted with Whatman No 1 filter paper disks treated with essential oil diluted in acetone. The filter paper was placed in a 9 cm diameter Petri dish. Three mL of different concentrations of *O. gratissimum* solutions were obtained by diluting 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 μL of pure essential oil in acetone and 350 μL of each solution sample was uniformly flowed on a 9 cm diameter disk of filter paper previously introduced into separate Petri dishes. The treated filter papers were left to dry at room temperature for 5 min after which 10 adult *S. zeamais* were introduced into each Petri dish and enclosed. A control without essential oil was run. Each treatment was replicated two times with four sub-replicates for each Petri dish along with control sets. After 24 h, insect mortality was assessed. The mortality rate was corrected for control response using Abbott's formula.

The experimental set up used for the crude essential oil test was also used to assess the remnant effect of the oil on grain. In this test 200 g of maize was mixed with 6 mL of *O. gratissimum* essential oil solution diluted in acetone. Concentrations used here corresponded to the LC_{95} found for crude essential oil. After the grain coating, acetone was left to evaporate for 15 min. The control included grain treated with acetone alone. Every 2 days, 20 g of maize grain was introduced in glass vials (diameter 2.5 cm, height 9.5 cm, volume 40 mL). The vials containing insects were kept under conditions used for testing the whole essential oils. Survival was recorded in four replicates, 24 h after treatment.

2.7. Preparation of clay-O. gratissimum essential oil formulation

Formulation used in this study was prepared with modified montmorillonite (Mont-CTMA) and unmodified one (Mont-Na) by using the following ratio

$$\frac{m_{EO}}{m_{clay}} = 0,1$$

with m_{EO}: mass of essential oil; m_{clay}: mass of clay.

To prepare 10 g of each formulation, 10 g of clay powder (Mont-Na or Mont-CTMA) were transferred in a 100 mL flask and the