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Studies on FT-IR Spectroscopy of modified Montmorillonite clays applied for the removal of T-2 toxin in maize

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Abstract-

Montmorillonite clay has a wide range of industrial applications which include the removal of mycotoxins in foods and feed because of its low toxicity both *in vitro* and *in vivo*. T-2 toxin is produced mostly by fungal species of *Fusarium*. Other T-2 producing fungal species are *Myrothecium* and *Stachybotrys*. T-2 toxin poses several health hazards such as dystrophy in the brain, heart, kidney and liver as well as ulceration and necrosis of the digestive tract in man and animals. To reduce T-2 toxin in maize, montmorillonite clay modified with lemongrass essential oil (MMT-LGEO) and montmorillonite clay washed with NaCl (Na-MMT) were applied to maize at a concentration of 8% and 12% and kept under storage for one month at 30°C. Untreated maize samples and unmodified montmorillonite clay (MMT) served as controls. The FTIR spectra were recorded for the two treatments and unmodified montmorillonite clay (MMT) used for the removal of T-2 toxin in maize. The FTIR spectra of the two treatments and unmodified clay (MMT) showed the major functional groups as Si-O and -OH. All the treatments reduced the level of T-2 toxin in maize. However, sodium montmorillonite (Na-MMT) and montmorillonite clay modified with lemongrass essential oil (MMT-LGEO) were more efficient than unmodified montmorillonite clay (MMT) in the removal of T-2 toxin in maize.

Keywords: FTIR, montmorillonite clay, removal, T-2 toxin

1. Introduction

Maize is grown worldwide, and the top three producers in the world are the United States, China, and Brazil, which produce approximately 563 of the 717 million metric tonnes/year [1]. However, mycotoxins, including T-2 toxins, contaminate cereals, especially maize. T-2 toxins are common in grains, which include oats, soybeans, rice, barley, maize and wheat [2]. T-2 toxin is produced mostly by fungal species of *Fusarium*, and other T-2 producing fungal species are *Myrothecium* and *Stachybotrys*. The presence of fungi in cereal grains could be as a result of the normal flora of the plants as well as contamination from the field or during storage and display for sales [3]. This toxin belongs to the family of mycotoxins known as the trichothecenes. Four types of trichothecenes exist namely: Type A, B, C and D. T-2 is a Type A trichothecene which is the most toxic group of trichothecenes. Generally, all trichothecenes have a common tetracyclic sesquiterpenoid 12,13-epoxytrichothec-9-ene ring system. They also possess an epoxide group located at their C12, C13 positions which is responsible for their



toxicity [4]. T-2 poisoning in humans results in alimentary toxic aleukia (ATA). The toxin causes ulcers as well as necrosis in the digestive tract, hemorrhagic inflammation and dystrophy in the brain, heart, kidney and liver; and the walls of blood vessels are damaged provoking hemorrhagic diathesis [5]. The toxin also affects poultry causing ulcerative and necrotic lesions as well as oral lesions, thereby leading to feed refusal [6]. T-2 toxin affects pigs causing alimentary toxic aleukia (ATA) with symptoms such as shock, haemorrhage, leukopenia, diarrhoea, vomiting and death.

Montmorillonite (MMT) clay is a type of smectite nanoclay which is abundant and environmentally friendly [7]. It has an octahedral sheet located between two tetrahedral sheets. Its silicon oxide tetrahedron (SiO_4) shares its 3 out of 4 oxygen atoms with the central octahedral sheets [7]. Clays have been employed in the removal of toxins from foods [8]. Plant extracts have also been employed in the decontamination of mycotoxins from food [9]. However, the combination of clay and plant extracts have not been employed in the decontamination of mycotoxins in foods. Hence, a decontamination strategy was developed for the removal of T-2 toxin in cereals using a combination of montmorillonite clay and extracts of *Cymbopogon citratus* (lemongrass) which is also naturally abundant.

2. Methodology

2.1 Sample Preparation

Montmorillonite K10 powder (CAS number 1318-93-0 (Sigma-Aldrich) was purchased while *Cymbopogon citratus* (lemongrass) leaves used for the study were identified in the Botany Unit of the Department of Biological Sciences, Covenant University, Nigeria. Sodium chloride salt was obtained from Sigma-Aldrich and samples were prepared by deionized water. T-2 toxin standard was obtained from Sigma-Aldrich (Bornem, Belgium).

Montmorillonite clay was modified with the crude essential oil of *Cymbopogon citratus* (lemongrass) using the method of [10] with slight modification. The other montmorillonite clay was modified by washing the clay with 1mM NaCl solution in the ratio 1:20. Montmorillonite K10 was used as a control. Fresh leaves of *Cymbopogon citratus* were gently washed with distilled water to remove dirt, and the leaves were allowed to air-dry at room temperature for three (3) weeks. The dried leaves were ground into powder by using an electric blender (IKA M20, USA). The soxhlet extraction method was used to obtain lemongrass crude extracts following the method of Ojewumi et al. [11].

2.2 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy of the clay samples was performed with the aid of a Perkin Elmer Spectrum 100 FTIR Spectrometer. One drop of Nujol (a liquid hydrocarbon) was added to 1 mg of each sample and mixed thoroughly. The mull was then placed between sodium chloride plates, and the spectrum was recorded.

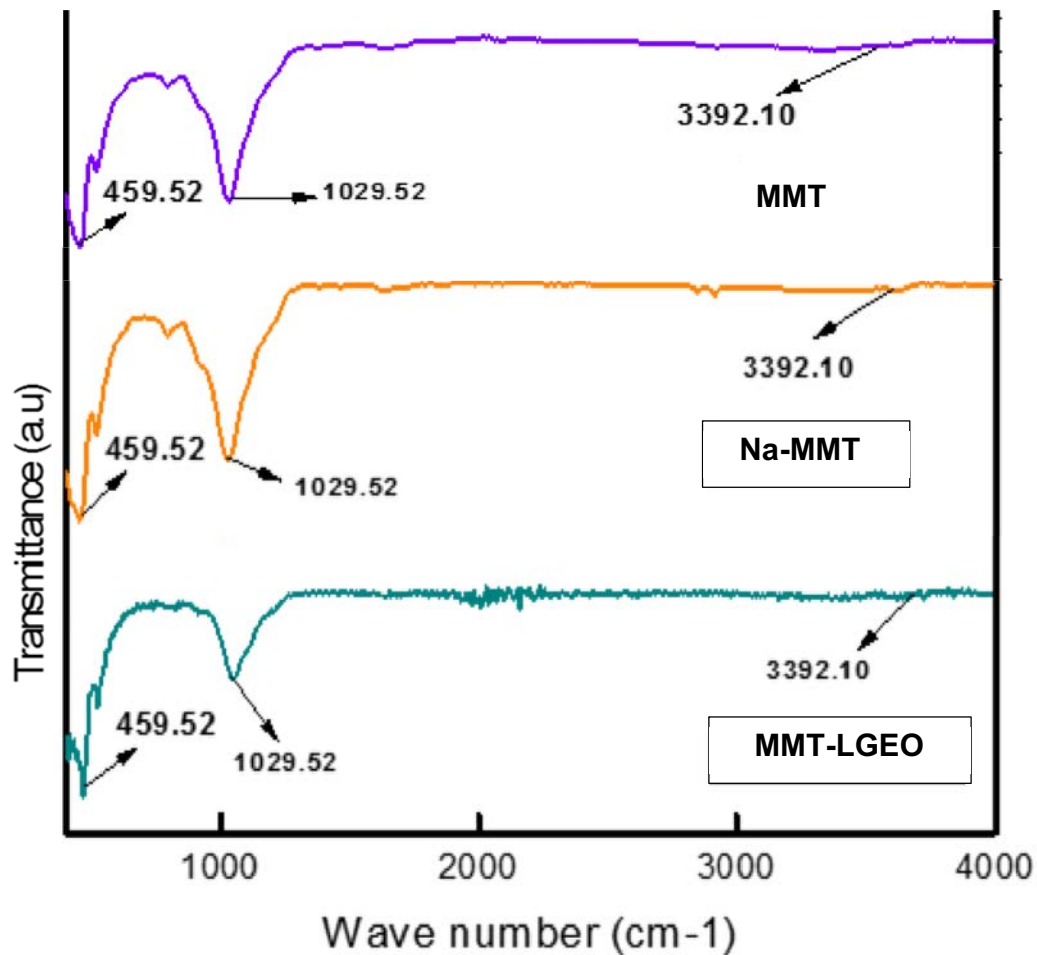


Figure 1: FTIR spectra of MMT, MMT-LGEO and Na-MMT

2.3 Application of Nanoformulations for the decontamination of T-2 toxin in maize

Liquid Chromatography-tandem Mass spectrophotometer (LC-MS/MS) as reported by Sulyok et al. [11] was used to quantify T-2 toxin in the maize sample before treatment with the two types of modified clay (MMT-LGEO and Na-MMT). Each treatment was applied to the maize sample in duplicates at 8% and 12% and stored for 4 weeks at a temperature of 30°C in the incubator following the modified method of [9]. A control was set up by subjecting the maize sample from the same batch to the same conditions of storage but without any treatment applied. The LC-MS/MS technique was used to determine the concentration of T-2 toxin in the maize samples treated with the two types of modified montmorillonite clay at the end of 4 weeks.

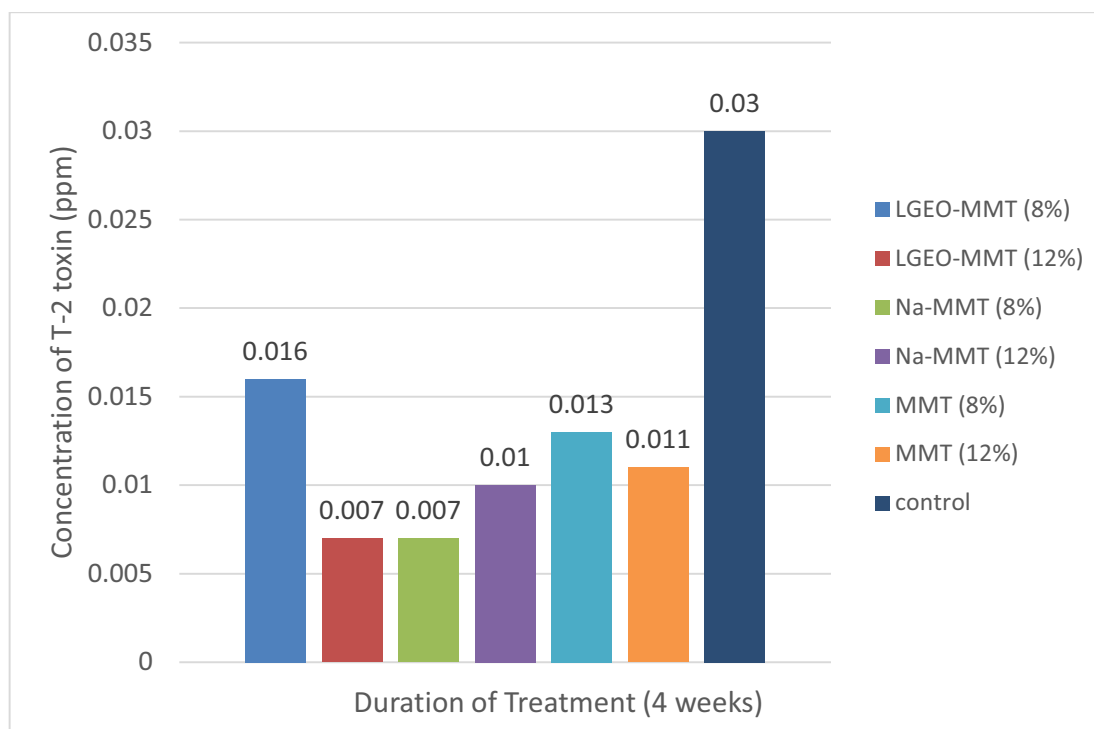


Figure 2: Level of T-2 removal after a treatment period of 4 weeks.

3. Result and discussions

3.1 Fourier Transform Infrared (FT-IR) Spectroscopy

The Infrared spectra of montmorillonite clay modified with lemongrass essential oil (MMT-LGEO) and sodium montmorillonite (Na-MMT) are shown in Figure 1. There were visible bands at 459.52 and 1029.52 cm^{-1} and a slight depression along 3392.10 cm^{-1} for both types of modified montmorillonite clay. The infrared (IR) spectrum of MMT-LGEO and Na-MMT, as shown in Figure 1 revealed prominent peaks at 459 and 1029 cm^{-1} , which represent the bands for Si-O-Si bending and stretching, respectively [13]. These bands were present in the two types of modified montmorillonite clay and the unmodified clay; MMT which served as the control. The peaks in the region 3392 cm^{-1} represent the stretching of the -OH (hydroxyl group) of water within montmorillonite [13]. The most prominent peaks were observed at 459 and 1029 cm^{-1} . This suggests that silicon and oxygen bonds play a role in the decontamination of mycotoxins, such as T-2 toxins.

3.2 The removal of T-2 toxin in maize

The two types of treatment (MMT-LGEO) and Na-MMT) applied to the maize sample from the same batch were effective over the treatment period of 4 weeks. The control (untreated maize stored under the same conditions as samples that were treated) was constant throughout the treatment period of four weeks, with a concentration of 0.03 ppm (Figure 2). T-2 is a Type A trichothecene and belongs to the group of polar mycotoxins. Sodium montmorillonite (Na-MMT) treatment at 8% was effective in the removal of T-2 toxin from 0.03 ppm to 0.007 ppm. Lemongrass essential oil-modified montmorillonite clay at 12% was also effective in the

removal of T-2 toxin at the same rate. Therefore, montmorillonite clay, which has high adsorptive properties along with the positive charge of sodium (Na^+) led to the effective adsorption of T-2 toxin from its negative polar ends. The presence of the lemongrass extract which has antifungal properties could have also contributed to the inhibition of fungal species responsible for the production of T-2 toxin, thereby causing a removal in the mycotoxin level while montmorillonite was efficient in adsorbing the toxin from the cereals.

4. Conclusion

Sodium montmorillonite (Na-MMT) and MMT were both efficient in the removal of T-2 toxin. The surface of montmorillonite clay was modified with a sodium (Na^+) ion which was able to trap T-2 toxins from its negative polar end. The MMT-LGEO was intercalated with organocations from the lemongrass extracts which were also responsible for trapping T-2 toxin from its negative polar ends.

5. Recommendation

Various plant extracts in combination with clay should be tested for the decontamination of T-2 toxin in cereals.

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