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Characterization of Nanoformulations from Montmorillonite clay for the decontamination of zearalenonein cereals using X-ray Diffraction Technique

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Abstract. Montmorillonite clay due to its abundance and environmental friendliness has several industrial applications among which are the adsorption of mycotoxins in foods and feed. The mycotoxin; zearalenone (ZEA) is oestrogenic and has been implicated in hormonal and reproductive issues for both man and animals. Thus, nanoformulations from Montmorillonite clay and *Cymbopogoncitratus* (lemongrass) extracts were developed for the reduction of the toxin in millet. The various formulations include: Montmorillonite clay to which extract of *Cymbopogoncitratus*(lemon grass essential oil)has been incorporated (Mont-LGEO), Montmorillonite clay mixed with *C. citratus*(lemon grass)powder (Mont-LGP) and Montmorillonite clay washed with NaCl (Mont-Na). Pure Montmorillonite clay (Mont) and *C. citratus* (lemon grass) powder (LGP) served as the controls for the treatment. Each of these compositions were applied to millet samples at a concentration of 8 % and 12% and stored for 4 weeks. XRD patterns for compositions containing Montmorillonite clay revealed major peaks at 2-Theta value of 20.06° representing Montmorillonite and 26.56° and 68.53°representing quartz. The level of ZEA in all treated samples was quantified after 4 weeks using the Liquid Chromatography tandem mass spectrophotometer LC-MS/MS. All the compositions were effective in the decontamination of zearalenone. Furthermore, *C. citratus* powder was the most effective in the decontamination of zearalenone in the cereal after 4 weeks.

Keywords:decontamination,quartz,XRD, zearalenone

1. Introduction

Mycotoxins are secondary metabolites produced by toxigenic fungi especially moulds. Most mycotoxins are detrimental to man and animals. For instance, aflatoxins are carcinogenic, Ochratoxins are nephrotoxic and Deoxynivalenol are immune suppressive [1].Of particular interest is Zearalenone which is referred to as amycooestrogen[1]. Zearalenone (ZEA) is an estrogenic compound produced by several different species of *Fusarium* such as *Fusariumgraminearum, F. culmorum, F. cerealis, F. equiseti, F. crookwellense* and *F. semitectum*[2]. ZEA is usually produced pre-harvest but can also be produced under extremely bad storage conditions especially high moisture content [3].Climate changes resulting in appropriate weather conditions aids the growth of *Fusarium* species; causing ear rot, seedling blight and stalk rot [4].

Zearalenonehas been identified as the cause of hyperoestrogenic syndromes experienced by humans [5]. Toxicological studies showed that ZEA affects the reproductive systemthereby leading to altered reproductive tract,decreased fertility,enlarged uterus as well as abnormal level of progesterone and estradiol. In view of this, a mycotoxin decontamination strategy was developed using Montmorillonite clay and extracts of *Cymbopogoncitratus*to reduce the level of this toxin in millet.

Montmorillonite (MMT) is a dioctahedral 2:1 phyllosilicate which is made up of two tetrahedral sheets and one octahedral sheet. Montmorillonite has an interlayer space between each triple-sheet-layer. Montmorillonite possesses a negative residual charge due to isomorphic substitutions in the tetrahedral sheet of Si^{4+} by Al^{3+} and Al^{3+} by Mg^{2+} in the octahedral ones[6].

2. Materials and methods

2.1 Materials

The Montmorillonite clay used in this study was Montmorillonite K10 powder (CAS number 1318-93-0) from Sigma-Aldrich while *Cymbopogoncitratus* (lemongrass) leaves were obtained from a farm in Ogbomoso, Oyo state. NaCl was also purchased from Sigma-Aldrich and deionized water was used for the preparation of the sample.

2.2 Sample Preparation

A nanocomposite was formulated using Montmorillonite clay and *Cymbopogoncitratus* (lemongrass) extracts according to the modified method of [7]. The second formulation was made by mixing Montmorillonite clay and the biomass of*Cymbopogoncitratus*in the ratio 1:1. The third formulation was Montmorillonite washed with 1mM NaCl in the ratio 1/20 (50g clay/1000mL NaCl). Unmodified Montmorillonite K10 served as the control as well as the dry biomass of *C. citratus* (lemongrass). Lemon grass was rinsed in distilled water to remove dust particles and air-dried at room temperature. They were pulverized with the aid of an electric blender (IKA M20, USA). Soxhlet extraction was done according to the method of [8] to obtain crude extract (essential oil) from lemongrass while some parts of the dry biomass of the lemon grass was reserved for mixture with unmodified Montmorillonite clay.

2.3 X-Ray diffraction

The X- ray diffractometer measurements were taken with the aid of the X'Pert PRO PW 3050/60 diffractometer. This was done with Cu-Kα radiation (n=1.54060Aº) at 40kv and 40mA. The scan range was through 4.0084º to 89.9774º with a step size of 0.0170º.

2.4 Application of Nanoformulations for the decontamination of Zearalenone in millet

The level of zearalenone in the millet was quantified using the Liquid Chromatography tandem Mass spectrophotometer (LC-MS/MS) as described by [9] before the application of nanoformulations. The formulations were applied to the cereals in duplicates at 8% and 12% and stored at 30 ºC for 4 weeks according to the modified method of [10].A millet sample from the same treatment batch was also subjected to the same storage condition as the treated samples over the same period to serve as a control. The level of mycotoxins in the treated millet with various formulations were analyzed after the storage period of 4 weeks using LC-MS/MS protocol described by [9].

3.Results and discussion

3.1 X-Ray Diffraction

The XRD patterns of Montmorillonite-Lemongrass essential oil, Lemon grass powder, Montmorillonite-Na, Montmorillonite and Montmorillonite-Lemongrass powder are shown in Figure 1. The diffractograms showed that quartz and montmorillonite were the major phases present in the samples. A peak was also observed at the 2 Theta value of 26.56º for lemongrass powder showing the presence of silica in lemongrass powder (LGP) which was also present in the other compositions containing clay (Figure 1).The XRD patterns of the various formulations with Montmorillonite clay had similar peaks but at varying intensities.

The XRD patterns of all the clay fractions displayed peaks for montmorillonite and quartz. The XRD patterns of the modified clays were very similar. The diffractograms showed that quartz and montmorillonite were the major phases present in the samples. There was no sharp peak present in lemongrass powder (LGP) showing that it is not crystalline in nature.However, the peak at 22º revealed the presence of amorphous silica (Fig. 1). The XRD patterns of the three modified Montmorillonite clays showed varying intensities in the peaks present. Only the peak for lemongrass powder was wide (Fig 1). The peak at 20.06° represents Montmorillonite while the presence of quartz was indicated by the peaks at 2 Theta value of 26.56° and 68.53°. The peaks listed above had higher intensities for the formulations containing lemongrass (Fig. 1). Quartz which is also known as silica showed a higher intensity compared with clay without lemongrass. This is because lemongrass also contains silica making the intensity higher than formulations without lemongrass extract. Hence the combination of Montmorillonite clay and lemongrass extract resulted in a more crystalline nanocomposite than formulations without the lemongrass extract.

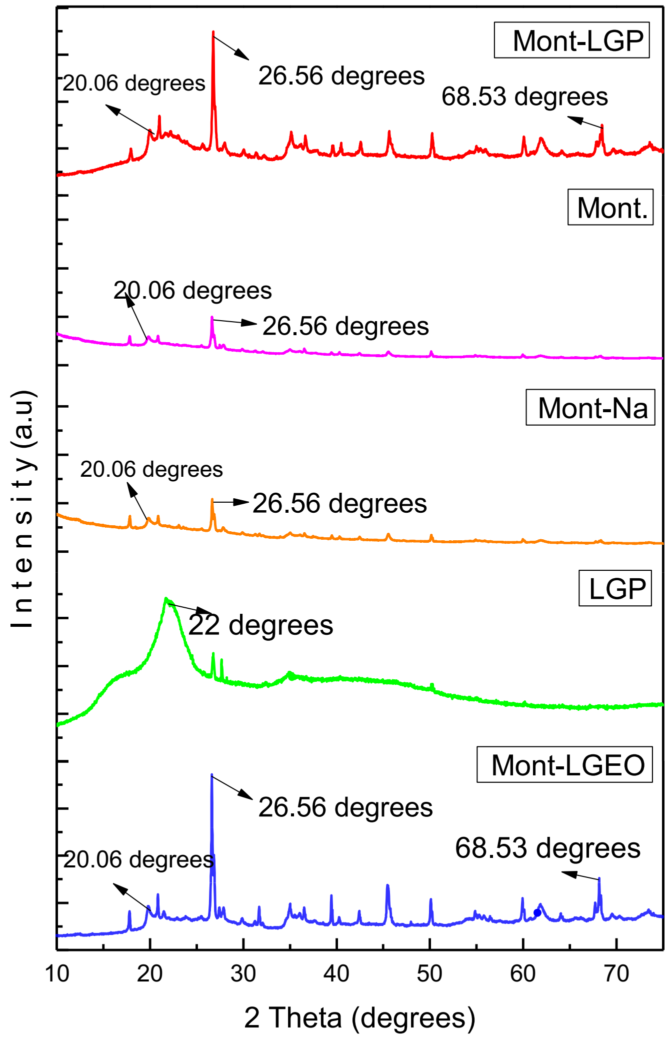

Figure 1:XRD patterns of Mont-LGEO, LGP, Mont-Na, Mont. and Mont-LGP

3.2 Evaluation of zearalenone reduction in treated millet

The various nanoformulations applied to the millet samples from the same batch were effective over the treatment period of 4 weeks. The control (millet without any formulations but kept under the same storage conditions as the treated samples) remained constant at the end of four weeks (Fig. 2).

Lemon grass powder (LGP) was the most effective treatment against zearalenone (ZEA) after 4 weeks.Lemon grass is rich in volatile oils which is responsible for its lemon flavor [11]. An example of this volatile oil is Citral, a major component which has been reported to be hydrophobic [12]. The presence of volatile oils in lemon grass contribute to its hydrophobicity. Zearalenone is also known to be a non-polar mycotoxin hence it is hydrophobic [13]. Therefore, the hydrophobic interaction of ZEA with the surface of Lemon grass powder was responsible for its efficient adsorption.

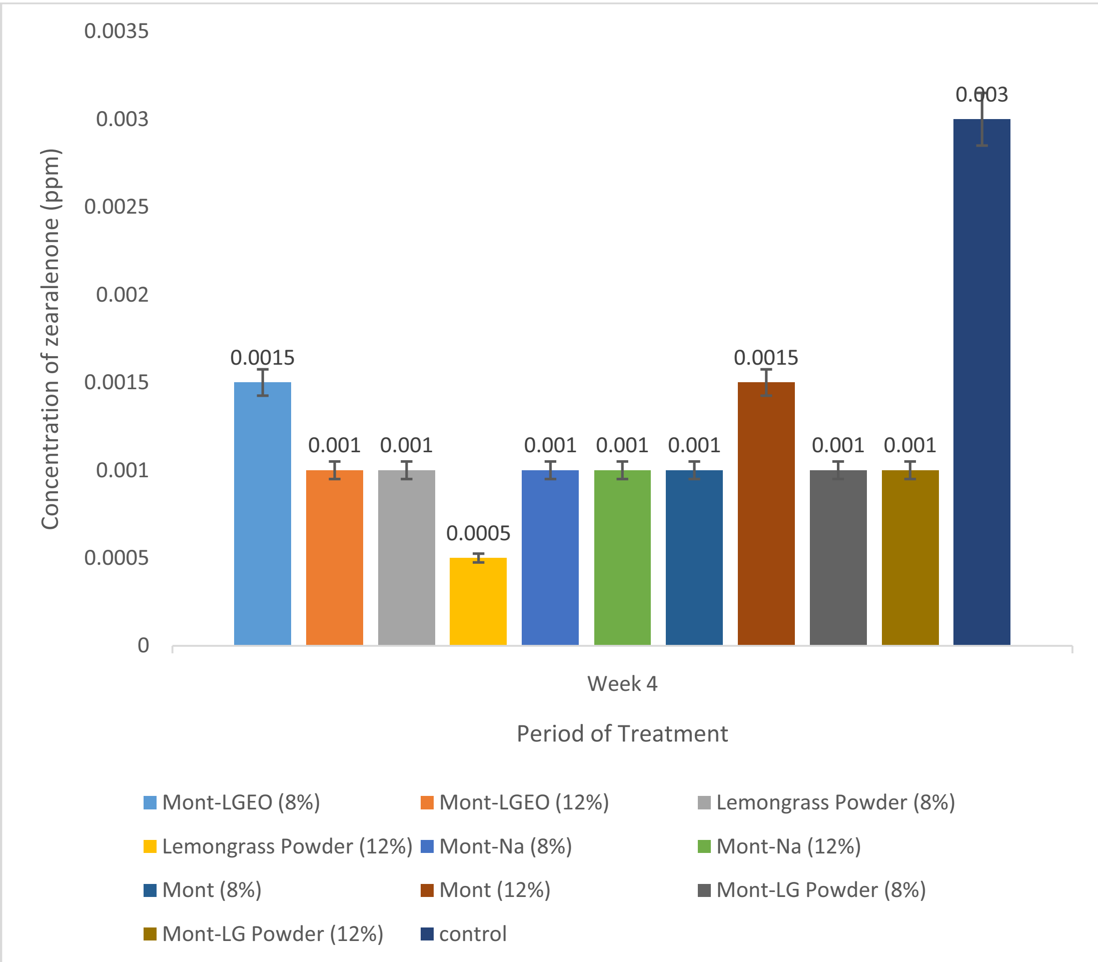

Figure 2: Evaluation of zearalenone level after treatment with nanoformulations for 4 weeks.

4. Conclusion

Lemongrass powder was the most effective for the decontamination of Zearalenone because of the hydrophobic interactions between its surfaces and ZEA. There were also marked changes in the surface properties of the montmorillonite clay containing plant extracts. The incorporation of an organo-cation from lemongrass makes the clay change from being hydrophilic to hydrophobic thus making it effective for the adsorption of ZEA which is also hydrophobic.

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