Biochar prepared from *Ficus nitida* as a carrier for frankincense essential oil (*Boswellia sacra*) to control some stored product insects

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**Abstract:** The insecticidal activity of biochar that prepared from *Ficus nitida* tree residues at 500 and 700°C was evaluated against some stored product insects *Tribolium castaneum*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis*, alone and as a carrier for the frankincense essential oil (*Boswellia sacra*) after 0, 15 and 30 days storage periods. The results showed the *O. surinamensis* was the most susceptible and the biochar prepared at 500°C was the most active against all tested insects. Also, the toxicity increased with increasing storage period only against *R. dominica*. The formula was more toxic than biochar or oil alone, especially against *T. castaneum*. The elemental analysis showed low carbon and high oxygen contents in the biochar 500 and the FTIR analysis showed a large number of functional groups on biochar 500 compared to biochar 700 which may attribute to the slightly higher toxicity of biochar. SEM images of the ventral surface of treated *O. surinamensis* showed the adhesion of biochar on all body parts, Moreover, the sensilla within the external surface of the elytra are partly absent. Our results suggest the promising use of biochar against some stored product insects and can be effectively loaded with other safe chemicals, more studies are needed to understand its effects on insects.

**Keywords:** biochar, *Ficus nitida*, essential oils, frankincense oil, *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*

**Introduction**

Because of its simple storage, handling, and transport, cereal grains became a serious source of staple food throughout the world. (Paul *et al.* 2020). Approximately, 24 percent of the entire food globe production loosed Post-harvest, and an outsized proportion of those losses was caused by insect infestation. Losses caused by insect infestations in grains, starting from about 9% to twenty in developed and developing countries respectively. (Phillips & Throne 2010). Various insect species are documented to attack Wheat and other stored goods which cause a reduction in the quality of the infested commodities, leading to both quantitative and qualitative losses (Pimentel 2002, Saad *et al.* 2018). The most key insect pests are those belonging to the Coleoptera order. Among these species, the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) is one among the foremost destructive insect species of cereals and legumes stored worldwide (Hagstrum & Phillips 2017, Hill 2002, Mason & McDonough 2011). The sawtoothed grain beetle, *Oryzaephilus surinamensis* it’s understood that pests target stored foods and it’s likely to be found in cereals and fruits that have been stored (Panagiotakopulu & Buckland 2017). *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) or the red flour weevil, maybe a universal insect species that causes extreme infestations of storage grains, various sorts of
flour, milled cereals, beans, nuts, etc. (Hagstrum & Phillips 2017, Hill 2002, Layek et al. 2022, Robinson 2005).

The widespread use of chemical insecticides in agriculture leads to many problems, ecotoxicological, economical, and social implications. These problems have led researchers to find more environmentally sustainable and viable alternatives than synthetic chemicals. The use of insecticides based on botanical extracts has attracted the attention of both researchers and consumers. The most promising alternative is Essential oils (EOs) which are used as insecticides due to their worldwide availability and relative cost-effectiveness (Campolo et al. 2018).

Biochar is a highly absorbent and porous carbon-rich material, similar in semblance to charcoal and other materials which are riches in carbon, but is purposely developed as an amendment to the soil. Biochar is a co-product of biomass pyrolysis obtained as a solid product from the exposure of organic materials to elevated temperatures under low oxygen conditions (anaerobic). Biochar can be generated from almost any form of feedstocking biomass (Aly 2016). A variety of natural elements, such as nitrogen, oxygen, phosphorus, and calcium, are found in Biochar. The composition of these elements is dependent on the nature of the treated source of the biomass (Alvarenga et al. 2016, Taherymoosavi et al. 2017). Also, the organic portion of the produced biochar is variable and depends on the composition of raw biomass. Due to its massive advantages and environmentally friendly nature (Das et al. 2020).

Through the carbonization process of biomass to produce biochar, the carbon (C) stabilized and can be stored for a long-time due to the relatively resistance of biochar to decomposition, therefore, biochar plays a significant role in carbon sequestration thus reduce the bad impacts of climate change (Ayaz et al. 2021; Cowie et al. 2017; Layek et al. 2022).

According to European Biochar Foundation, biochar is feed able and edible as result of being non-toxic (EBC 2012). The gradual addition of biochar to feed, silage, bedding material revealed that biochar can be applied sequentially. Due to its high adsorptive capacity for different toxins, several scientific and clinical studies revealed that one or more positive effects can be achieved when biochar was supplied as feeding for livestock (Schmidt et al. 2019).

In a study conducted to investigate the effects of biochar on the structure and diversity of gut microbial communities in Wistar rats which fed orally on rice straw biochar (RSB) for 5 weeks at a dosage of 1120 mg/kg of body weight and found that RSB enhanced the structure of gut mucosal and improved epithelial integrity. Moreover the bacterial community was improved due to RSB addition, which may positively affect the growth and gut vitality in rats (Han et al. 2018).

Nowadays, researchers find the use of biochar compounds as eco-friendly alternatives to replace conventional pesticides. (Sayed et al. 2018). Also, (Cook & Andrade 2018) studied the direct impact of one dry biochar formulation on the survival of various typical forest insect species the insects showed high mortality when had direct contact with the biochar. Biochar has been developed in resolving many environmental problems such as adsorbing pollutants (Saravanan et al. 2018). Thus, the distinctive chemical and physical properties have corresponding effects on oil absorption. According to (Kandanelli et al. 2018), biochar may be utilized as an oil adsorbent material with a high adsorption capacity of 2–3 g oil/g biochar without any physical or chemical modification. So, biochar can absorb essential oils and promote its permanence and effect to protect stored grains from insect and fungal infestation for long periods (Huang et al. 2021, Edenborn et al. 2018).

Little attention had been focused on the influence of biochar as a biopesticide for
stored product insects’ management. Insecticidal impacts of biochar are not well known (Bakhat et al. 2020).

In this work, we have evaluated for the first time the effectiveness of biochar prepared from the remnants of the *ficus nitida* tree at two different temperatures 500 and 700°C to control different types of stored product insects. Also, the effectiveness of frankincense essential oil prepared from *Boswellia sacra* that loaded on the same type of biochar was evaluated in comparison with the effectiveness of the biochar alone.

**Materials and methods**

**Tested insects**

The red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the lesser grain borer *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and the sawtoothed grain beetle *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae) were cultured in the laboratory at 28°C ±1, r.h. 70 ±10 and photoperiod L/D 12:12 h over than six years at the Faculty of Agriculture, Alexandria University. *T. castaneum* was reared on a mixture of 90% whole wheat and 10% brewer’s yeast (wt/wt) as described by (Beeman et al. 2009) *R. dominica* were reared on whole wheat according to the method explained by (Kavallieratos et al. 2005). While, *O. surinamensis* diet of whole wheat flour, rolled oats and yeast (5:5:1) according to the methods of (Watson & Barson 1996).

**Preparation of biochar**

Biochar was prepared from pruning residue of *ficus nitida*. Trees are grown in the forestry research sector of Antoniades botanical garden, Alexandria. The pruning branches were air-dried in the open air for about three months after that the branches were stored in the lab at room temperature. The branches were debarked and sawn into suitable pieces. Wood samples were placed in crucibles, covered with a tightly fitting lid and pyrolysis under oxygen-limited conditions in a muffle furnace. The pyrolysis temperature was raised to 500°C or 700°C at approximately 15°C/min and held for 60 min. Then, the biochar was allowed to cool to room temperature, ground and stored in sealed bags until use.

**Biochar characterization**

Scanning electron microscopy (Jeol JSM-5300 SEM) was used for the observation of biochar surface microstructure and SEM with electron dispersive X-rays analysis (EDX) was used to analyze the content of the element at acceleration voltage 15-20KeV. The identification of the distribution of functional groups on biochar surface, Fourier transform infrared (FT-IR) technique used in the range of 400-4000 cm⁻¹. Burker tensor 37 spectrometers were used by the technique of KBr pellet as 1.0 mg of samples was added to 100 mg KBr pressed, then exposed to infrared radiation (Wu et al. 2012, Guo &Chen 2014). The pH values of both biochar samples were measured at a ratio of 1:20 (w/v) in water after being shaken for 24 h at 200 rpm (Zheng et al. 2013).

**Essential oil**

Frankincense *Boswellia carterii* essential oil obtained from Sakara essential oils’ company Giza, Egypt.

**Analysis of essential oil**

Analyses of essential oil were carried out on gas chromatography-mass spectrometry (Hewlett Packard 5890) couplet with MS engine 5989B in the EI mode was used for compounds identification. Injected samples were diluted into diethyl ether and 1ml, and injected 1µl. The GC was equipped with on an Rtx-5MS, solid-phase 5% diphenyl-95%dimethyl polysiloxane capillary column 30mx0.25mm, 0.25µm. The GC conditions were as follows: the temperature program: 50°C (2°) to 250°C for 10 minutes, with a rate of 8°C/min, helium flow rate 1ml/min. A Thermo Finnegan GC-MS equipped with an Rtx-5MS (30mx0.25mm, 0.25µm) column was also used.
in similar conditions. The GC/MS interface line and the ion source were maintained to 200°C or 250°C. Electron energy was 70eV and electron emission 100μA. RES. Chemical compositions of frankincense essential oil are listed in Table (1).

Biochar bioassay technique
A series of concentrations of both biochar *ficus nitida* 500 and 700 in the range of 0.5 - 2.5g/kg. Each concentration was applied with 1 kg sterilized wheat in a 1.5-liter glass jar. Each jar covered with a piece of Clingfilm ® covers. Jars were then manually tilled (10x) and rotated for 2 minutes, with a brief interval of shaking at 120s. After shaking, the jars were kept closed for a few minutes to allow any loose dust to settle. Then, the wheat was placed in plastic bags (silo bags) and kept for 15, 30 days in each jar. Then, 20 g of each treated wheat were weighed and infested with twenty adults of each insect in different times of period storage (0, and 15 days and 30 days). Each concentration was replicated three times. Mortality percentages were recorded after 10 day exposure period for both biochar 500 and 700 (Subramanyam & Roesli 2000).

Frankincense oil and BFO bioassay technique
In glasses of 0.25 L, a series of concentrations of frankincense oil was dissolved in acetone (1 ml) and applied to 20 g of sterilized wheat. Allow 15 minutes for the solvent to evaporate entirely in the jars; the control was treated with acetone alone. A series of concentrations of BFO formula weight and applied to 20 g of sterilized wheat in a 0.25 L glasses jar. The jars were continually agitated for 10 minutes to ensure a uniform distribution of biochar throughout the wheat surface. Twenty adults of *T. castaneum*, *R. dominica*, and *O. surinamensis* were inserted in each jar after a 15-day storage period. Each concentration was replicated three times in a glass jar with tight plastic closure. The jars were incubated in a lab condition. Mortality percentages were recorded after 10 day exposure period for both oil and BFO. Toxicity of frankincense essential oil against *T. castaneum*, *R. dominica* and *O. surinamensis* after storage period of 15 days were presented in the Table 5.

Insect scanning electron microscopy
Untreated and treated insects of *O. surinamensis* were collected after the end of experiment and fixed by immersing them immediately in 4F1G (Fixative, phosphate buffer solution) PH=7.4 at 4 °C for 3 hours according to methods of (Tahmasebi et al. 2015). Specimens were then postfixed in 2% OsO4 in the same buffer at 4°C for 2 hours. Samples were washed in the buffer and dehydrated at 4C through a graded series of ethanol. Insects were dried by means of a critical point method, mounted using carbon paste on an AL- stub and coated with gold up to a thickness of 400 A in a sputter–coating unit (JFC-1100 E). Observations of insect morphology in the coded specimens were performed in a Jeol JSM- 5300 scanning electron microscope operated between 15 and 20 KeV at the Faculty of Science; Alexandria University.

Data analysis
LC50 values and their upper and lower confidence interval limits, as well as intercept, were estimated by probit analysis were calculated according to (Finny 1971) using Ldp line software (Ehab Soft, Cairo, Egypt). Non-
overlapping, 95% fiducially limits were used to determine significant differences among treatments (P<0.05). Co-toxicity coefficients were calculated following (Sun & Johnson 1960).

Results

Biochar characterization

Electron dispersive X-ray (EDX) image of biochar prepared at 500°C. (The part of highlight with a red circle was scanned). EDX indicates an element of carbon and oxygen are most abundant elements. And, having traces of elements both potassium and calcium Fig. 1. As well, EDX shows an increase in carbon and decrease of oxygen mass percentage in biochar 700 than biochar 500 Fig. 2. Scanning electron microscopy (SEM) images of biochar produced at 500°C contain fine-sized granules Fig. 3. The biochar samples prepared at 700°C contain fine-sized granules in a greater proportion than those prepared at 500°C Fig. 4. The analysis of FTIR for the biochar that was prepared at two different temperatures 500°C and 700°C as shown in Fig. 5 and Table 1 confirms the results of elemental analysis. The biochar prepared at 500°C showed more functional groups than that prepared at 700°C. The biochar 500 was with a pH value of 6.6, the biochar 700 with a pH of 7.8.

Fig. 1. Electron dispersive X-ray (EDX) image of biochar prepared at 500°C. (the part of highlight with red circle was scanned).

Fig. 2. Electron dispersive X-ray (EDX) image of biochar prepared at 700°C. (the part of highlight with red circle was scanned).
Fig. 3. Scanning electron microscopy (SEM) images of biochar derived from *Ficus nitida* produced at 500°C.

Fig. 4. Scanning electron microscopy (SEM) images of biochar derived from *Ficus nitida* produced at 700°C.

Fig. 5: the FTIR spectra for biochar prepared at pyrolysis temperatures 500 °C (-) and 700°C (-).
Table 1. FTIR band and corresponding function groups for biochar prepared at both temperature.

| Wavenumber (cm\(^{-1}\)) | Assignments                      | 500 \(^{\circ}\)C | 700 \(^{\circ}\)C |
|--------------------------|----------------------------------|-------------------|-----------------|
| 3000-3700                | -OH group                         | 3598.5078         | 3699.3041       |
|                          |                                  | 3520.7306         |                 |
|                          |                                  | 3441.6396         |                 |
| 2800-3000                | C-H (methyl)                      | 2922.6780         | -               |
|                          |                                  | 2854.0948         |                 |
| 1600-1800                | C=O /COOH                         | 1698.3416         | 1872.8632       |
| 1560                     | C=C                               | 1567.5455         | -               |
| 1420-1450                | C-H asymmetric                    | 1429.3678         | 1441.1250       |
| 1317-1375                | C-H bending (symmetric and asymmetric or C-O asymmetric of aromatic) | 1352.0043         | -               |
| 1000-1260                | C-O                               | 1105.9327         | 1123.8248       |
| 700-900                  | C-H aromatic (out of plane)       | 877.3988          | 875.9336        |
|                          |                                  | 805.3937          |                 |
| 400-700                  | In organic mattar                | 561.4482          | 465.9102        |

Chemical composition of frankincense oil

The chemical component and relative content of the total frankincense oil were shown in Table 2. Among them, the main components of frankincense oil were alpha-Thujene (30.70%), octyl acetate (20.44%), cis-Z-à-Bisabolene epoxide (9.76%), 9-3, 3-dimethylxirian-2-yl) -2, 7-dimethylnona-2, 6-dien-1-ol (8.46%). As well, 1R-à-Pinene, p-Xylene, Sabinene, 3-Carene, Limonene, o-Cymene, Estragole, Verticillol and Oxirane were existed.

Table 2. Chemical composition of the Frankincense essential oil (Boswellia carterii) under investigation.

| S. no. | Compound a                  | RT b     | Area % |
|--------|-----------------------------|----------|--------|
| 1      | alpha-Thujene               | 4.99     | 30.70  |
| 2      | 1R-à-Pinene                 | 5.20     | 2.88   |
| 3      | -2Pentanone, 4-hydroxy-4-methyl-1,5-diphenyl- | 5.37     | 0.85   |
| 4      | p-Xylene                    | 5.71     | 2.51   |
| 5      | Sabinene                    | 6.14     | 2.11   |
| 6      | Pinene                      | 6.32     | 0.31   |
| 7      | -3Carene                    | 6.75     | 1.62   |
| 8      | Limonene                    | 7.25     | 2.96   |
| 9      | beta-Ocimene                | 7.67     | 0.92   |
| 10     | O-Cymene                    | 7.87     | 3.01   |
| 11     | Pentylcyclopropane          | 8.85     | 1.26   |
| 12     | Octyl acetate               | 13.18    | 20.44  |
| 13     | Terpinen-4-ol               | 13.34    | 0.57   |
| 14     | Estragole                   | 14.96    | 1.58   |
| 15     | alpha-Bourbonene            | 16.62    | 0.47   |
| 16     | Nerolidol                   | 22.55    | 0.78   |
| 17     | (-)beta-Elemene             | 31.02    | 0.81   |
| 18     | Verticillol                 | 33.12    | 1.24   |
| 19     | Oxirane                     | 35.71    | 4.73   |
| 20     | cis-Z-à-Bisabolene epoxide  | 35.77    | 9.76   |
| 21     | 9-3, 3-dimethylxirian-2-yl)-2,7-dimethylnona-2,6-dien-1-ol | 36.04    | 8.46   |
| 22     | 9-Eicosyne                  | 36.46    | 0.23   |
| 23     | Oleyl Alcohol               | 37.41    | 0.34   |
| 24     | cis-Z-à-Bisabolene epoxide  | 38.10    | 0.33   |
| 25     | Z-8-Methyl-9-tetradecenoic acid | 52.24    | 0.72   |

A: components of Boswellia carterii essential oil; b: Retention time. The main components of EO were alpha-Thujene (30.70%), octyl acetate (20.44%), Farnesyl acetate (9.76%), 10, 11-Epoxyfarnesol (8.46%).
Toxicity of biochar

The toxicity of biochar 500 against three tested insects, *T. castanum*, *R. dominica* and *O. surinamensis* adults after different storage periods 0, 15 and 30 days have been summarized in Table 3. The toxicity of biochar increased with increasing storage time against *R. dominica* 0.75, 0.56 and 0.39 after 0, 15 and 30 days. Unlike the other, *T. castaneum* and *O. surinamensis* the toxicity decreased with time of storage. The results obtained from Table 4 presents the efficacy of biochar 700. The toxicity of biochar increases over time storage for both *T. castanum* and *R. dominica*, where LC$_{50}$s were more than 45, 10. 30, 3 and 1.32, 0.51, 0.48 against *T. castaneum* and *R. dominica*, respectively. In general, biochar 500 frankincense essential oil showed more effectiveness than biochar 700. The insecticidal efficiency of frankincense essential oil was evaluated after a storage period of 15 days. Results in Table 5 indicate that frankincense oil represented highly effective against *O. surinamensis* where LC$_{50}$ was 0.64, followed by *T. castaneum* LC$_{50}$ was 4.41. However, *R. dominica* showed less sensitivity LC$_{50}$ was 8.38 g/kg. We examined a new formula based on frankincense oil and biochar 700 to enhance the toxic efficiency of the biochar 700 Table 6. The formula was more toxic than biochar or oil alone against *T. castaneum*, *R. dominica* and *O. surinamensis* LC$_{50}$s were 0.35 and 0.85 g/kg with Co-toxicity factor-based biochar were 3.77 and 3.20.

Table 3. Efficacy of the pyrolysis temperature 500°C on biochar *ficus nitida* toxicity against *T. castaneum*, *R. dominica* and *O. suranaminsis* at different storage periods.

| storage periods | Type of insects   | LC$_{50}$ (g/kg) | Confidence limits | Slope± variance | ch$^2$ | P-value |
|-----------------|-------------------|------------------|-------------------|-----------------|--------|---------|
|                 |                   |                  | lower             | upper           |        |         |
| 0 days          | *T. castanum*     | 3.60a            | 2.30              | 11.04           | 1.19±0.27 | 3.52    | 0.06    |
|                 | *R. dominica*     | 0.75b            | 0.26              | 1.19            | 0.73±0.25 | 0.01    | 0.94    |
|                 | *O. suranaminsis* | 0.41             | 0.13              | 0.58            | 1.24±0.29 | 2.01    | 0.15    |
| 15 days         | *T. castanum*     | > 20             | -                 | -               | -       | -       |
|                 | *R. dominica*     | 0.56             | 0.30              | 0.77            | 1.16±0.27 | 0.27    | 0.63    |
|                 | *O. suranaminsis* | 0.73             | 0.35              | 1.05            | 0.89±0.26 | 0.14    | 0.71    |
| 30 days         | *T. castanum*     | > 29             | -                 | -               | -       | -       |
|                 | *R. dominica*     | 0.39             | 0.14              | 0.58            | 1.08±0.27 | 0.50    | 0.48    |
|                 | *O. suranaminsis* | 1.00             | 0.47              | 1.78            | 0.71±0.25 | 0.51    | 0.49    |

Table 4. Efficacy of the pyrolysis temperature 700°C on biochar *ficus nitida* toxicity against *T. castaneum*, *R. dominica* and *O. suranaminsis* at different storage periods.

| storage periods | Type of insects   | LC$_{50}$ (g/kg) | Confidence limits | Slope± variance | ch$^2$ | P-value |
|-----------------|-------------------|------------------|-------------------|-----------------|--------|---------|
|                 |                   |                  | lower             | upper           |        |         |
| 0 days          | *T. castanum*     | > 45             | -                 | -               | -       | -       |
|                 | *R. dominica*     | 1.32             | 0.93              | 2.18            | 0.97±0.26 | 0.11    | 0.74    |
|                 | *O. suranaminsis* | 2.72             | 2.19              | 3.83            | 2.22±0.32 | 0.01    | 0.98    |
| 15 days         | *T. castanum*     | 10.30            | 4.80              | 113.82          | 1.27±0.35 | 1.95    | 0.16    |
|                 | *R. dominica*     | 0.51             | 0.16              | 0.77            | 0.89±0.26 | 0.01    | 0.92    |
|                 | *O. suranaminsis* | 4.60             | 2.40              | 70.02           | 0.80±0.27 | 0.95    | 0.33    |
| 30 days         | *T. castanum*     | 3.00             | 1.98              | 8.20            | 1.09±0.27 | 0.01    | 0.95    |
|                 | *R. dominica*     | 0.48             | 0.08              | 0.78            | 0.74±0.26 | 0.03    | 0.86    |
|                 | *O. suranaminsis* | 4.46             | 2.28              | 117.12          | 0.74±0.27 | 0.90    | 0.34    |
Table 5. Toxicity of frankincense essential oil against *T. castaneum*, *R. dominica* and *O. suranaminsis* after storage period 15 days.

| Type of insects | LC50 (g/kg) | Confidence limits | Slope ± Variance | ch² | P-value |
|----------------|------------|-------------------|------------------|-----|---------|
| *T. castaneum* | 4.41       | 3.28 - 6.96       | 1.37 ± 0.19      | 5.97| 0.05    |
| *R. dominica*  | 8.38       | 4.51 - 39.52      | 0.76 ± 0.18      | 2.14| 0.34    |
| *O. suranaminsis* | 0.64     | 0.5 - 0.77        | 1.95 ± 0.23      | 2.27| 0.32    |

Table 6: Toxicity of biochar *ficus nitida* 700°C formula based frankincense essential oil against *T. castaneum*, *R. dominica* and *O. suranaminsis* after storage period 15 days.

| Type of insects | LC50 (g/kg) | Confidence limits | Slope ± Variance | ch² | P-value | Co-toxicity factor based biochar |
|----------------|------------|-------------------|------------------|-----|---------|----------------------------------|
| *T. castaneum* | 1.42       | 1.29 - 1.55       | 3.61 ± 0.32      | 1.63| 0.44    |                                 |
| *R. dominica*  | 0.35       | 0.27 - 0.57       | 1.78 ± 0.29      | 1.72| 0.42    | 3.77                            |
| *O. suranaminsis* | 0.85     | 0.64 - 1.04       | 1.49 ± 0.24      | 5.87| 0.05    | 3.20                            |

* Co-toxicity coefficients were calculated following Sun and Johnson (1960)

**Scanning electron microscopy**

The Scan Electron Microscopy (SEM) photographs of the *O. surinamensis* adults treated with biochar 500 compared with the untreated insects are illustrated. The Scan Electron Microscopy (SEM) photographs of the *O. surinamensis* adults treated with biochar 500 compared with the untreated insects are illustrated. The SEM micrograph Fig (6a) of a whole-body ventral surface view of untreated *O. surinamensis* adults shows a clear waxy surface of the cuticle. Untreated insect shows waxy cuticle of the elytrum tegument on the dorsal surface, which have pores with whole small and large sensilla fig (6b). On the other hand, the view of the insects treated with biochar shows biochar completely glued on all body parts fig (7a). Moreover, the sensilla within the external surface of the elytra are partly hidden or absent by the act of biochar fig (7b). Furthermore, Fig (7c) shows particles of the biochar covering almost all of elytra pores.

![Fig 6](image1.png)  
**Fig 6:** SEM photographs of the *O. surinamensis* adults (untreated) shows the ventral surface (a) and the dorsal surface (b).

![Fig 7](image2.png)  
**Fig 7:** SEM photographs of the *O. surinamensis* adults treated with biochar shows completely glued on all body (a), the absence of sensilla (b) and elytra pores that covered with biochar (c).
Discussion

Several studies demonstrated the effect of biochar on soil insects. (Bakhat et al. 2020) concluded that, the rice husk biochar tends to reduce insect infestation on brinjal plants by improving Si uptake. (Meilin & Rubiana 2018) also reported that rice husk biochar reduced the soil insects in the potato grown field. Moreover, (Waqas et al. 2018) stated that rice varieties amended with rice husk biochar indicated higher resistance against white-backed rice hoppers. Our results indicated the higher activity of biochar prepared at the lower temperature, this activity may result from the effect of pyrolysis temperature on the chemical composition of biochar surface and that partially obtained by the results of EDX analysis as there is a noticeable increase in the percentage of oxygen in the samples prepared at a temperature of 500, and decrease in the percentage of carbon, which is present in the composition of many active groups that may be dissociated at temperature 500 compared to those prepared on biochar 700 degrees and this results was confirmed by the FTIR analysis. The biochar 500 was acidic however biochar 700 was alkaline because of the high content of organic matter or many active groups in biochar 500 and increase of oxygen mass percentage and decrease of carbon mass percentage unlike biochar 700. This explains the effective action of biochar 500 was more than biochar 700. Overall, temperature preparation biochar is affected by its physical properties (carbon and oxygen and other elements, oil absorbency, pH value, particle size) that are correlated to their insecticidal efficacy against stored-product insects. Whenever pyrolysis temperature increased pH, basic functional groups, and total content of C, and Ca while acidic functional groups decreased (Al-Wabel et al. 2013). Our results were in agreement with those obtained by (Borgohain et al. 2020, Usevičiūtė & Baltrénaitė-Gedienė 2020) which found the amount of C increased as the pyrolysis temperature raised from 300 to 700 °C, but the amount of H, O, and N decreased. Also, biochar was still effective after a storage period of 30 days. Maybe this is due to the physical structure of biochar and its ability to absorb and retain moisture from grains which caused insects cannot able to infect grains (Abit et al. 2012). The toxic effects of frankincense oil could be attributed to some well-known toxic constituents such as alpha-Thujene (30.70%) is classified as a monoterpene that acts as neurotoxicants against many insects (Kiran et al. 2017). The frankincense oil was found to be rich in Octyl acetate ester (20.44%), which is the main component of the many effective essential oils (Tabanca et al. 2012). In our experiments, we observed a synergistic effect on the toxicity of biochar and tested essential oil when administered together. The combined of biochar 700 and frankincense oil against T. castaneum was highly significantly compared with biochar 700 alone after 15 days storage period. The Co-toxicity factor of formula based biochar was high effective 3.77, 3.20 against R. dominica and O. surinamensis. The increased toxicity of essential oil when mixed with biochar might be due to a slower release and, as a result, a higher persistence of essential oil volatiles when adsorbed onto the surface of biochar particles (Islam et al. 2010). Over and above the small particles and their structures that constitute the biochar, whose large surface area maybe improve the essential oil retention. Previous research has found that a combination of essential oil and inert dust had a longer-lasting insecticidal effect than the two substances used separately (Pierattini et al. 2019, Khorrami et al. 2018, Yang et al. 2010).

SEM photographs of the O. surinamensis adults treated with biochar 500 shows completely glued on all body and critical damage of all body parts. The particles of biochar close up the pores of the insect cuticle; the sensilla were damaged or hidden (Wahba and Attia 2019, Malia et al. 2016). Biochar is a material that is abrasive to an insect’s cuticle,
increasing the possibility of dehydration (Cook & Andrade 2018). It might be getting into the insect's spiracles and affecting or interfering with the respiration mechanism. Furthermore, biochar action particles are known to degrade hydrocarbons in nature, therefore it possibly degrades alkanes which present in insect cuticles (Ahmad et al. 2014).

Conclusion

Our findings suggest that biochar might be a promising and effective methodological approach for the integrated management of these stored product insects and that this innovative valorization method could be an addition to the others that have been offered previously. In this aspect, a biochar frankincense oil compound has shown to be effective against T. castanum, R. dominica, and O. surinamensis. In the framework of the Circular Economy, wastes derived from agriculture might be utilized to create innovative bioinsecticides. Over the succeeding years, more research will be performed to assess the activity and potential utility of various biomass pyrolysis biochar as sources of insecticidal compounds.

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