T.C.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

TOXIC AND BEHAVIORAL EFFECTS OF SOME INERT DUSTS ON *SITOPHILUS GRANARIUS* L. (COLEOPTERA: CURCULIONIDAE)

MUSA SÜRÜCÜ

July 2020

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MASTER THESIS

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Master Thesis

Supervisor

Prof. Dr. Ayhan GÖKÇE

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The study titled "**Toxic and Behavioral Effects of Some Inert Dusts on Sitophilus granarius L. (COLEOPTERA: CURCULIONIDAE)**" are presented by **Musa SÜRÜCÜ** under supervision of **Prof. Dr. Ayhan GÖKÇE** has been accepted as Master Thesis by juries, at the Department of **Plant Production and Technologies,** Niğde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.

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THESIS CERTIFICATION

It is certified to "***Toxic and behavioral effects of some inert dusts on Sitophilus granarius L. (Coleoptera: Curculionidae)***" thesis by ***Musa SÜRÜCÜ*** were written based on principals of Niğde Ömer Halisdemir University - Graduate School of Natural and Applied Sciences, Department of Plant Production and Technologies. It is also certified that, all information and sources used in this thesis were cited and referenced definitively, by author.

Musa SÜRÜCÜ

ÖZET

BAZI İNERT TOZLARIN *SITOPHILUS GRANARIUS* L. (COLEOPTERA: CURCULIONIDAE)'a TOKSİK VE DAVRANIŞSAL ETKİLERİ

SÜRÜCÜ, Musa
Niğde Ömer Halisdemir Üniversitesi
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Bitkisel Üretim ve Teknolojileri Anabilim Dalı

Danışman: Prof. Dr. Ayhan GÖKÇE

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Bu çalışmada, inert tozların (bentonit, halloysit, nobleit, kaolin, sepiyolit ve zeolit) *Sitophilus granarius* L. üzerindeki toksik ve davranışsal etkileri laboratuvar koşullarında araştırılmıştır. Çalışmadaki inert tozların tek doz (2000 ppm) tarama testinden öldürücülüğü en yüksek kontak toksisite, 120 saatte % 100.00 ölüm oranı ile nobleit ve kaolinde gözlenmiştir. Çalışmanın ikinci kısımında, yüksek etki gösteren nobleit ve kaolin ile doz-etki çalışması yürütülmüştür. Nobleit için LC_{10}, LC_{25}, LC_{90} değerleri 380, 875 ve 12788 ppm olarak hesaplanmıştır. Kaolin için ise LC_{10}, LC_{25}, LC_{90} değerleri 360, 618 ve 3574 ppm olarak bulunmuştur. Letal zaman (LT_{50}), nobleit için 169.64 ve kaolin için 148.86 saat olarak hesaplanmıştır. Davranışsal etki çalışmalarında, uzaklaştırıcı etki için inert tozlar ve kontrol arasında fark bulunmamıştır. Beslenmeyi engelleyici etkide ise nobleit % 81.49 etki ile diğer inert tozlardan istatistiki olarak daha etkili bulunmuştur. Yumurta bırakmayı engelleyici testlerde ise hem kaolin (% 67.15) hem de nobleit (% 59.00) etkili bulunmuştur. Elde edilen veriler, nobleit ve kaolin inert tozlarının *S. granarius* üzerinde toksik ve davranışsal etkisi olduğunu göstermekte olup, ileri düzeyde yapılacak çalışmalar ile tarımsal mücadelede kullanılma potansiyelini tam olarak ortaya çıkartacaktır.

Anahtar sözcükler: İnert tozlar, nobleit, kaolin, *Sitophilus granarius* L., toksik ve davranışsal etki

SUMMARY

TOXIC AND BEHAVIORAL EFFECTS OF SOME INERT DUSTS ON *SITOPHILUS GRANARIUS* L. (COLEOPTERA: CURCULIONIDAE)

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Toxic and behavioral effects of some inert dusts (bentonite, halloysite, nobleite, kaolin, sepiolite, zeolite) were studied against *Sitophilus granarius* L. under laboratory conditions on wheat grains. Single dose screeening test was carried out at 2000 ppm for each inert dust. Kaolin and nobleite caused 100.00% mortality after 120 hours of incubation. In the second series of experiments, dose-response study was carried out with kaolin and nobleite. LC_{10}, LC_{25}, LC_{90} values were calculated as 380, 875 and 12788 ppm and 360, 618 and 3574 ppm respectively for nobleite and kaolin. Lethal time (LT_{50}) was calculated 169.64 hours for nobleite, 148.86 hours for kaolin at LC_{25} dose. Behavioral effect of nobleite and kaolin were also studied. Repellent effect of inert dusts were insignificant as compared with the control group. Antifeedant effect of nobleite was calculated as 81.49 % as it was 43.16% for kaolin. Nobleite and kaolin produced antiovipositional effect with 59.00% and 67.15% inhibition rate, respectively. These results reveal that, nobleite and kaolin have some toxic and behavioural effects on *S. granarius* adults under tested conditions and further studies at different enviromental conditions are needed to explore the full potential of these inert dusts in controlling of the granary weevil populations.

Keywords: Inert dusts, nobleite, kaolin, *Sitophilus granarius* L., toxic and behavioral effect

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SYMBOLS AND ABBREVIATIONS

Symbols	**Descriptions**
ml	Milliliter
pH	Quantity of hydrogen ions
µl	Microliter
%	Percent
°C	Degree centigrade
RH	Relative humidity
TM	Trademark
®	Registered trademark
mm	Millimeter
cm	Centimeter
m^2	Square meter
mg	Milligram
g	Gram
kg	Kilogram
ppm	Parts per million
t	Ton
d	Day

Abbreviations	**Descriptions**
DE	Diatomaceous Earth
LCn	Sufficient amount of concentration to kill “n” percent of population
LDn	Sufficient amount of dose for killiing “n” percent of population
LTn	Required time for killing “n” percent of population
FAO	Food and Agriculture Organization
TMO	Turkish Grain Board

TAGEM	General Directorate of Agricultural Research and Policies
BKÜDB	Database of Plant Protection Products
IPCS	International Programme on Chemical Safety
DPT	State Planning Organization

CHAPTER I

INTRODUCTION

Cereals including wheat, barley, triticale, rice, millet, sorghum, maize, oat, and rye crops are always considered as indispensable with their vital role in human nutrition and livestock feeding since beginning of the agriculture (McKevith, 2004). They are generally easy to grow in large area within various geographical regions. They have played important roles in the development of human civilizations. Evidences of their role have been excavated from different parts of the World as well as in Turkey (Awika, 2011).

Wheat (*Triticum aestivum* L. Poaceae: Poales) is one of the staple cereal due to its nutritional value and has better storage advantages as comparison to other crops, come from its physiological and biochemical availability (Grundas and Wrigley, 2016). Humidity, temperature and other parameters are important and relatively easy to store wheat grains for long periods (Doblado et al., 2012). In 2018, the world wheat production was approximately 735 million tons 21.5 million tons of which were produced by Turkey (TMO, 2018). Beside all these advantages, availabilities and high production amount, cereals are affected by many abiotic and biotic factors which cause significant grain losses during storage period (Mishra et al., 2012; Dizlek, 2012).

Abiotic factors, temperature, humidity, drought and salinity conditions have various effects during growth and storage periods of cereals. The abiotic factors also have effects on the biotic factors in many ways (Abhinandan et al., 2018). Biotic factors include fungi, bacteria, viruses, insect pests and so on that cause direct or indirect damage to grains (Hernandez Nopsa et al., 2015). One of the most important biotic factor in wheat production is insect pests (USDA, 2015). They damage and reduce quality of wheat grains during growing period in field (e.g. sunn pest) as well as in storage period (e.g. weevils) (Hagstrum et al., 1999). Therefore, wheat production and storage period of it have serious insect pest problems.

Until harvest time, there are several insects feed on and damage wheat plants and their parts, including roots, glumes, stems, petioles, leaves and grains. The main insects are wheat aphids, (*Rhopalosiphum padi* (L.), *Diuraphis noxia* (Kurdjumov)), *Sitobion avenae* (F)), thrips (*Anthothrips tritici* (Kurdjumov)), sunn pests (*Eurygaster* sp.), wheat stinkbugs (*Aelia* sp.), scale insects *Porphyrophora tritici* (Bodenheimer) and so on (TAGEM, 2008).

In storage conditions, *Tribolium castaneum* (Herbst) (red flour beetle), *Tribolium confusum* Jaquelin du Val. (confused flour beetle), *Trogoderma granarium* Evert. (khapra beetle), *Cryptolestes ferrugineus* (Stephens) (rusty grain beetle), *Oryzaephilus surinamensis* (L.) (saw toothed grain beetle), *Sitophilus oryzae* L. (rice weevil), *Sitophilus granarius* L. (granary weevil), *Tenebrio molitor* L. (mealworm beetle), and *Rhyzopertha dominica* (F.) (lesser grain borer) are the main insect pest species in many regions of the World (Kumar and Kalita, 2017) and in Turkey (Işıkber et al., 2014). Although there are some management practices, huge yield losses and nutritional reductions take place due to insect pest infestations (Kalsa et al., 2019). Depending on climate, control measures and storage type, this infestation leads to 30-40% physical damage or quality loses (Kumar and Kalita, 2017).

Sitophilus granarius L. (Coleoptera: Curculionidae) is one of the stored product pests which infests stored cereal grains, especially wheat, barley and oat. Studies on *S. granarius* damage on cereals revealed that, grains are directly affected by *S. granarius* and this effect is more destructive than other storage pests excluding species in the same genus (Golebiovska, 1969). Campbell and Sinha (1976) tested feeding behaviors of *S. granarius*, *C. ferrugineus* and *R. dominica* and evaluated their effects on wheat grain weight losses. The greatest grain weight loss (69.2%) was caused by *S. granarius* within one month (larvae and adult stages) and that was significantly higher than weight losses caused by *C. ferrugineus* (4.5%) and *R. dominica* (23.3%). Although there are not many studies focusing on yield loss caused by *S. granarius*, infestation and yield loss risks can be extremely high in uncontrolled and traditional storages in Turkey (Bağcı et al., 2014).

Management practices of stored product pests including *S. granarius* have limitations. Cultural and preventative measures against the insects are insufficient to eliminate quantity and quality risks, they can infect cereals during storage period and even in packages (Bell, 2011). Therefore, chemical control is more widely adapted to control the stored product pests especially by fumigation. In Turkey, the fumigation of stored products are implemented by using three main active substances (aluminum phosphide, magnesium phosphide and phosphine). Additionally, insecticides can be applied to eliminate any ongoing insect infestation before storing wheat grains by using chlorpyrifos-methyl and deltamethrin active substances (BKÜDB, 2020). However, intensive use of these insecticides leads to increase the risk of insect resistancy. As an evidence of this risks, studies show that *S. granarius* are resistant to many compounds including phosphine and deltamethrin (Monro et al., 1972; Kljajić and Perić, 2006). Another concern about chemical control measurement is their direct effect on human health. Such as, phosphine affects mitochondrial cells of liver and inhibits respiratory chain mechanism in rats at laboratory experiments (Sciuto et al., 2016). Additionally, there are several poisoning cases related with aluminum phosphide and phosphide based fumigants that are used in storages as a tablet form (Sharma, 1995; Tolunay et al., 2017; Terece et al., 2019). Phosphine and metal phosphides are also toxic to aquatic organisms, wild birds and mammals (IPCS, 1988).

In the literature, there are many biological and bio-rational management studies against the stored product pests under laboratory conditions. They show that, coleopteran storage pest populations including *S. granarius* can be successfully reduced or suppressed by these products (Phillips and Throne, 2010; Upadhyay and Ahmad, 2011). However, there are some disadvantages of biological agents, microbial substances and plant-based products' usage because they are relatively impracticable comparing with conventional insecticides under storage conditions (Schöller et al., 1997; Martynov and Brygadyrenko, 2019).

Using alternative control methods like inert dusts become more important because they do not cause or have limited toxicity and residual activity to environment (Golob, 1997). In this way, inert dusts and clays can be useful tools for management of *S. granarius*.

Inert dusts are clay, non-clay and soil based ash materials, derived from living and non-living organism fossils, which have potential of insecticidal activity. This insecticidal activity was known by Aztecs many years ago. However, main studies and developments were carried out after 1950s including diatomaceous earth (DE), kaolin and zeolite materials (Golob, 1997). These materials are rarely adapted to agricultural areas but they are extensively used in petroleum, paper and fertilization industries. Composition, texture and color of them show variation due to their property ratio and formation phases. Therefore, classification and their use in various areas depend on their physical and chemical properties (Murray, 1991).

Generally, inert dusts affect storage pests by desiccation and water absorption from insect cuticle causing insect de-hydration, therefore it is considered as physical control method (Ebeling, 1971). Using inert dusts can be a promising alternative especially for organic and integrated pest management practices by allowing lower environmental risk and residue free crops. There are some other advantegous of using inert dusts in food industry. For example, inert dusts can be directly applied to the grains and then can be removed easily from by washing. They can also be used in packages of products for protecting products from pest risks during storage period (Quarles, 1992; Golob, 1997).

Due to above-mentined pest problems in storages and insufficiency of control methods, unique and alternative control measurements should be investigated. With this purpose, the main objective of this study is formed to evaluate the toxic and behavioral effects of bentonite, halloysite, nobleite (calcium borate hydrate), sepiolite, kaolin, and zeolite inert dusts against *Sitophilus granarius* L. on shelled (aşurelik) wheat grains.

CHAPTER II

REVIEW OF LITERATURE

Mewis and Ulrichs (2001) tested Fossil Shield® (73% SiO_2) DE against several storage pests including *Sitophilus granarius*, at 25 °C at eleven different relative humidity (RH) (0, 10, 20, 32, 43, 50, 60, 71.5, 80, 90 and 97.5%) conditions both in the media of fed with and without wheat grains. Control group and DE treated groups were analyzed for their antifeedant effect and mortality ratio. In both conditions, toxic effect of DE on *S. granarius* decreased with increasing RH, and weight loss were negatively correlated with the increase of RH.

Athanassiou et al. (2004) tested the effect of Insecto® (86.7% SiO_2), SilicoSec® (92% SiO_2), and PyriSec® DE formulations on oat, rye, and triticale against *Sitophilus oryzae* and *Tribolium confusum* adults. Doses used were 0.75, 1, and 1.5 g of DE/kg of grain. Grain-dose formulations were assessed at 26 °C and 60% RH. After 48 hours (h), mortality rate of *S. oryzae* were significantly lower on oat and rye than was on triticale. SilicoSec® was significantly effective than Insecto®, PyriSec®. After 168 h, mortality ratio of *S. oryzae* were not significantly different according to dose rate, applied media and commodity. After 48 h, *T. confusum* mortality ratio was significantly lower in oat and rye than that in triticale, and there were no significant difference depending on material and doses. After 168 h, mortality ratio of *T. confusum* was significantly lower in oat than rye and triticale. Doses were significantly different and SilicoSec® had significantly higher mortality ratio than Pyrisec® and Insecto®.

Baldassari et al. (2004) tested Protector® (DE) against *T. castaneum, R. dominica* adults through wheat and maize kernels under 23 °C and 34 °C. Preliminary dose of Protector® was determined as 0.25 g 500 g^{-1}, and applied on wheat grains for *Rhizopertha dominica* and determined 0.2 g 40 g^{-1} on maize kernels for *T. castaneum*. It was found that, Protector® implementation at 23 °C and 34 °C provided almost 100% mortality to the larvae of *T. castaneum.* Results for *R. dominica* adults were 74.03% mortality at 23 °C and 53% mortality at 34 °C. As a conclusion, Protector® achieved high mortality on larvae of *T. castaneum* and achieved relatively lower mortality at higher temperatures against *R. dominica* adults.

Athanassiou et al. (2005) tested efficacy of DE (Silicosec®) against *S. oryzae* and *T. confusum* at different concentration rates, temperature and time intervals. Doses were determined as 0.25, 0.5, 1 and 1.5 g/kg of wheat for both tested insects at 22, 25, 27, 30 and 32°C. Mortality results were also bounded by exposure intervals as 24 and 48 h to 7 days. It was resulted that, long intervals have higher mortality with increasing temperature. However, for *T. confusum*, mortality at 32°C was lower than at 30°C. Silicosec® was more effective against *S. oryzae* than *T. confusum*. 1 and 1.5 g/kg doses gave adequate mortality ratios against both storage pests.

Al-Iraqi and Al-Naqib (2006) tested insecticidal efficacy of rocky dusts against several stored pests. Ninitive, kaolinite, montmorillonite and bentonite dusts were used on wheat grains at four concentrations, 0.1, 0.2, 0.4 and 0.8% (w/w), at 33 ± 1 ºC and 60 ± 5% RH, against four common storage pests: *T. confusum, T. granarium, Oryzaephilus surinamensis* and *R. dominica* adults. For all materials, LC_{50} and LC_{95} were calculated. Ninitive dust was more effective that other inert dusts and LC_{95} values was calculated as 0.30, 1.17, 0.20 and 0.19% for *T. confusum*, *T. granarium*, *O. surinamensis* and *R. dominica*, respectively. Bentonite was the least effective dust and LC_{95} values were 3.00, 11.30, 0.68, and 2.80% respectively for mentioned insects.

Collins and Cook (2006) studied on efficacy of dry and slurry forms of two DEs (SilicoSec® and Diasecticide^{TM} (83.68% SiO_2)) on *S. granarius* adults. They were treated with doses of 5, 10 and 20 g/m^2, on applying without grains Petri dishes with incubating at 25 °C and 70% RH in darkness. SilicoSec® caused mortality ranging between 82–97%. There were no significant difference between mortalities observed in dry and slurry forms of SilicoSec®. Diasecticide^{TM} treatments were ineffective and produced less than 6% mortality at tested dosages against *S. granarius*.

Kavallieratos et al. (2010) tested efficacy and adherence ratio of three DE formulations (Protector®, SilicoSec®, and Insecto®) on three different durum wheat varieties (Athos, Pontos, Sifnos) against three important storage insect pests' adults (*R. dominica, S. oryzae and T. confusum*). Implementations were retained at three different dose levels (250, 500 and 1000 ppm). It was concluded that, experiments done on Pantos variety shown less effectiveness as comparison to Athos or Sifnos, against tested insects on mentioned doses. Hence, to achieve high mortalities of *T. confusum* it was required to more time intervals and high dose levels than *R. dominica, S. oryzae* for each varieties. Although lower adherence ratio were observed in Athos variety, general adherence ratio were between 90.3% and 95.3% for all materials and on all varieties in the study.

Kljajić et al. (2010) tested zeolite (Minazel® and Minazel Plus®) (63-68% SiO_2) and DE (Protect-It® (83.7% SiO_2)) formulation doses against *S. oryzae, R. dominica, T. castaneum* at 24°C ± 1°C and 45% ± 5% RH on wheat grains. Dose rates were arranged as 0.25, 0.50 and 0.75 g/kg for zeolite. DE recommended dose rates were arranged as 0.15 g/kg for *S. oryzae*, 0.20 g/kg for *R. dominica* and 0.30 g/kg for *T. castaneum.* It was resulted that, efficacy of DE was higher than zeolite formulations for all test insects excluding zeolite higher dose rates. While Minazel® and DE caused 100.00% mortality in 14 days, Minazel Plus® caused 94.00% mortality at the highest dose (75 g/kg) on *S. oryzae.* Efficacy of DE and zeolite were parallel to *T. castaneum* but with lower mortality ratios. The lowest mortality ratio was observed in *R. dominica,* and DE were significantly more effective than zeolite formulations.

Shams et al. (2011) evaluated Silicosec® concentrations against adults of *Calllosobruchus maculatus* and *S. granarius* on cowpea and wheat grains, respectively. After preliminary tests, concentration were applied as 250, 323, 426, 562 and 750 mg kg^{-1} for *S. granarius* and 300, 340, 387, 439 and 500 mg kg^{-1} for *C. maculatus*. Mortality ratios were calculated 24, 36 and 48 h later for *S. granarius*; 24 and 48 h later for *C. maculates*. For 24 h, the values of LC_{50} and LC_{95} were 351.55 mg/kg and 673.80 mg/kg for *C. maculatus*, 1512 mg/kg and 8454 mg/kg for *S. granarius*, respectively. For 48 h, the values of LC_{50} and LC_{95} were 299.92 mg/kg and 504.41 mg/kg for *C. maculatus*, 404.24 mg/kg and 1050 mg/kg for *S.granarius*, respectively. It was concluded that, insecticidal effect of Silicosec® has high potential to control of *C. maculates* rather than *S.granarius*.

Keszthelyi and Pál-Fám (2012) tested effect of Hungarian DE (Diatosec®, 70-80% SiO_2) against *S. granarius* on barley and maize grains. Dose rates were prepared as 1, 2 and 4 g kg^{-1} for barley and maize grains. Mortality ratio and number of progenies were calculated. It was resulted that, there was significantly differences between grain types. After 13 days of exposure, insects that feed on treated barley grains were achieved 13%, 37% and 67% mortality ratios, at the dose of 1, 2 and 4 g kg^{-1}, respectively. For maize grains, results were 0.00%, 2.41% and 5.13% at the dose of 1, 2 and 4 g kg^{-1}, respectively. Progeny production mean of *S. granarius* on maize grains were 8.66, 7.50 and 1.83 at the dose of 1, 2 and 4 g kg^{-1}, respectively. On barley grains, results were 1.50, 0.83, and 0.50 at the dose of 1, 2 and 4 g kg^{-1}, respectively. These results indicated that, there were also correlation in the progeny production with mortality ratio on grain types. *S. granarius* had more emerged adults on maize grains than barley grains at all dosages.

Lupu et al. (2015) tested three Romanian local DE (Pătârlagele, Urloaia and Adamclisi) efficacy, comparing to PyriSec® as a standard of experiment on *S. granarius* under laboratory conditions. Preliminary dose, at 900 ppm (900 g/t seeds) was applied. It was resulted that, the mortality ratio of Pătârlagele source was 96%, Urloaia source was 83.33%, Adamclisi source was 83.33% and PyriSec® was 100% at 7 days.

Ertürk et al. (2017) tested two different DE (Protector® and DEA-P (DE and Abamactin)) against *S. oryzae* adults at 30°C and 75% RH on Osmancık-97 cultivar of rice. Protector® mortality ratio results at 250, 500, 750, 1000, 1500, 1750 and 2000 ppm (i.e., mg DE/kg rice) were calculated during 7 days interval, until one month. After 7 days, at doses of 1500, 1750 and 2000 ppm mortality ratios were between 93% and 99% which were significantly effective than other doses. At 750, 1000 ppm, mortality ratios were between 73 and 78%. At 500 ppm mortality ratio was 23%, for 250 ppm it was 1.5%.

Liska et al. (2017) tested several inert dusts (Celatom® MN51, Croatian inert dust PD, Zeolite Slovakia(1, 2, 3), Zeolite Serbia, Zeolite Cabsorb ZS500A, Odorlock Natural Zeolite, Bentonite, KaMin™ 80, DOT(disodium octaborate tetrahydrate), Sipernat® 50 S and Aerosil® 200) to examine their insecticidal efficacy against *S. oryzae* at different rates (100, 200, 300 and 500 ppm) on wheat grains. It is resulted that, after 7 days of exposure, Celatom® MN51, Zeolite Slovakia, Zeolite Serbia and DOT achieved 92% to 100.00 % mortality ratios at 500 ppm. Bentonite, KaMin™ 80 inert dust had lower effect (27% to 41%) at 500 ppm as comparison to other inert dusts. Inhibition rate of inert dusts was calculated at 500 ppm. Celatom® MN51, Croatian inert dusts PD, Zeolite Slovakia 1, and DOT inhibition rates were between 82% to 88%, which means effective for reducing progeny. Bentonite and KaMin™ had lowest two of inhibition rate between 46% and 49%.

El-Aziz and El-Ghany (2018) tested anti-feedant and coherence effect of native Egyptian DE and its modifications against adults of *S. granarius* in different time interval exposure. These modifications (Al-DE, Ca-DE, Na-DE) were evaluated at 26 °C ± 2 and 70-80 RH. Doses were determined as 1.5, 1.0, 0.75 and 0.375 g kg^{-1} wheat grain. It is found that, efficacy of Al-DE and Ca-DE modifications is significantly effective than DE and Na-DE formulation in antifeedant experiments. However, coating effect of DE, Al-DE, Ca-DE and Na-DE not significantly different each other.

Gultekin et al. (2018) studied on DEs obtained from different deposits in Turkey together with two commercial DEs, Silicosec® (Biofa AG- Germany) and Desect® (Ep Naturals America) against *C. maculatus* adults on chickpeas. Evaluated concentrations were 100, 300, 500, 1000 and 1500 ppm. The local DEs were coded as BGN, BHN, AG2N, AC2N, CB2N, CCN, FB2N. Results showed that, most effective DEs after 1 day of exposure were CCN, AG2N and BHN causing 75%, 59%, 58% mortalities, respectively at 1500 ppm concentration. Silicosec®, Desect®, BGN, AC2N were applied at 1500 ppm concentration and produced 98-100% mortality after 7 days of exposure. The CCN, BHN, AG2N and CB2N caused 97-99% reduction in progeny (F1) production of *C. maculatus*. In conclusion, three Turkish DEs (CCN, AG2N and BHN) were highly toxic to *C. maculatus* after 7 days of exposure in comparison with commercial DEs Silicosec® and Desect®.

Alkan et al. (2019a) studied on DEs efficacy, obtained from domestic sources in Turkey (Turco 000, 004 and Turco 020), and used against larvae and adult of *T. molitor* at four different rates (0, 0.001, 0.002, 0.003 and 0.004 mg/cm^2) on wheat bran. For adults, LC_{50} and LC_{90} values were 0.006 and 0.019 g/cm^2 for Turco 000; 0.013 and 0.022 g/cm^2 for Turco 004; and 0.053 and 0.041 for Turco 020. Calculated LC_{50} for *T. molitor* larvae were 0.014, 0.034 and 0.032 g/cm^2 for Turco 000, 004 and 020, while LC_{90} values were 0.053, 0.089 and 0.075 g/cm^2, respectively. It can be concluded that, tested local DEs of Turkey have promising effect to *T. molitor*.

Alkan et al. (2019b) tested efficacy of Turkish native DEs (Turco000, Turco004, and Turco020) and commercial DE (Protector®) on chickpeas, against *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae). Six different concentrations (100, 200, 400, 600, 800 and 1000 ppm) were arranged and applied to determine progeny activity and toxicity effect at 25 ± 1 °C and 65 ± 1% RH. It is resulted that, Turco000 was achieved 100.00 % mortality ratio at the dose of 200, 400, 600, 800 and 1000 ppm in 7 days after treatment. Turco004 were achieved 100.00% mortality ratio at the dose of 400, 600, 800 and 1000 ppm in 7 days after treatment. Turco020 was less effective than other DEs which achieved 100.00% mortality ratio at the dose of 800 and 1000 ppm. New progeny and emerged insects were analyzed for calculation of inhibition rate. Protector® and Turco000 had almost 100.00% inhibition rate in all concentrations. Inhibition rate of Turco020 achieved 100.00% at the dose of 200, 400, 600, 800 and 1000 ppm. Turco004 achieved 100.00% at the dose of 600, 800 and 1000 ppm. It can be concluded that, tested local DEs of Turkey have promising effect to *A. obtectus* on chickpea.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

The main materials used in the study were *Sitophilus granarius* L. (Coleoptera: Curculionidae) and inert dusts bentonite, halloysite, kaolin, nobleite, sepiolite, and zeolite. Protector® and PyriSec® commercial diatomaceous earths were used as standard inert dust formulations (Figure 3.1).

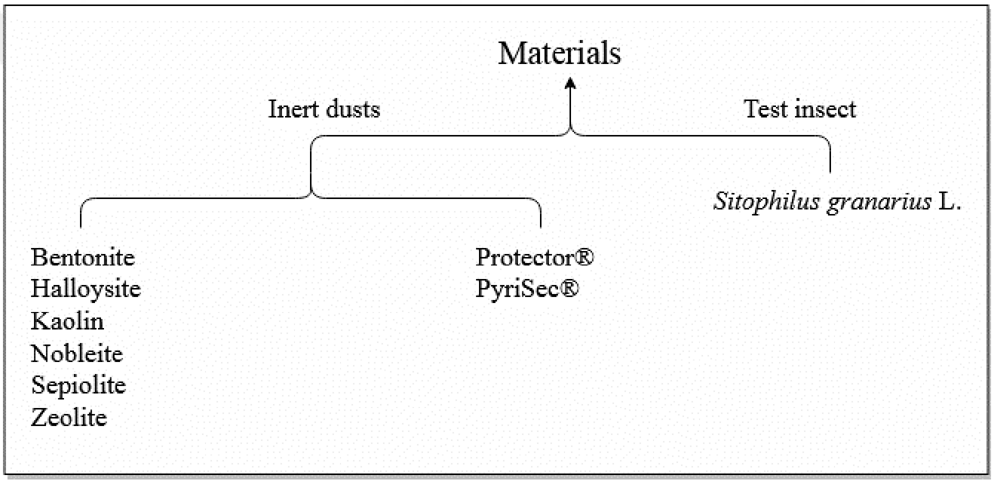

Figure 3.1. Scheme of materials used in experiments

3.1.1 *Sitophilus granarius* L. (Coleoptera: Curculionidae) (Granary weevil)

3.1.1.1 Taxonomy

Scientific name of *Sitophilus granarius* L. has changed several times. Firstly, named as *Curculio granarius* in 1758 by Linnaeus. In 1798, genus name was changed to *Calandra*, which included most of the weevils. The name of *Sitophilus* genus was introduced by Schoenherr and accepted in 1959 (Plarre, 2010). Finally, Delobel and Grenier (1993) introduced the last version of scientific name, as *Sitophilus granarius* L. which was modified from previous studies.

Phylum: Arthropoda

Order: Coleoptera

Family: Curculionidae

Genus: *Sitophilus*

Species: *Sitophilus granarius* L.

3.1.1.2 Description and biology

Sitophilus granarius L. adults are brownish-black insects, can be tawny-brown and reddish during their early life. They have characteristically long rostrum and total body size generally can vary between 3 to 5 mm. Mandibles are plate but narrowed through insertion area. Eight-segmented geniculate antenna are located at the base of rostrum. Wings has formed as elytra. Differently from other storage pests, *S. granarius* have non functional flight muscles so they cannot fly (Figure 3.2) (Bunescu et al., 2008; Plarre, 2010).

Figure 3.2. Dorsal (a) and ventral (b) surface of *Sitophilus granarius* L.

Common yield losses could be seen in wheat storages but the granary weevil can feed on rye, triticale, rice, oat, and also on sunflower seeds. Female adults lay their eggs into grains; egg, larval and pupal developments are completed inside grains (Figure 3.3). Generally, each grain has one egg. Even if a grain has more than one egg, only one insect can emerge due to larval cannibalism. They preferably do not reproduce in highly broken and milled grains like grits and flour (Mason and McDonoug, 2012).

Figure 3.3. Development process of *Sitophilus granarius* L. inside wheat grains

Female fertility of *S. granarius* depends on temperature and food availability. Approximately, one female can lay 1-8 eggs in one day and produce totally 150 eggs in its life span. Quality of eggs decreases in elder insects and new insects cannot emerge from these grains (Boniecki et al., 2020). Size of eggs are very tiny and hard to see without a microscope but larger than the eggs of *S. oryzae* (Lecato and Flaherty, 1974).

Figure 3.4. Larvae (a), (b) and pupae (c), (d) of *Sitophilus granarius* L.

As seen in Table 3.4, the size of larvae is approximately 1.6-3.5 mm and has creamy-whitish color with brown color head. They stay inside grains and pass through four instars (Mason and McDonoug, 2012). Pupate form has developed appendages like legs and head parts, including rostrum and antenna. Size of a pupa is almost as long as the last instar larvae but generally larger than all first three instars. Development of adults are also directly bounded by pupate developments, which emerge from hole that chewed inside out.

Damage of *S. granarius* is related to abiotic and biotic factors. At optimum conditions, they can complete their life cycle from egg to adult in 40 days. However, in undesirable conditions this cycle takes longer time. Concordantly, main yield losses caused by *S. granarius* is recorded in Middle East, Middle Europe, temperate regions in Asia, America and Australia (Plarre, 2010).

Sitophilus granarius L. is also a common stored product pest in Turkey. For example, Kütahya (Zengin and Karaca, 2020), Ankara (Bağcı et al., 2014), Adıyaman and Diyarbakır (Bolu, 2016) provinces and their countryside storages are highly infested with *S. granarius,* causing qualitative and qauntative losses during storage and packaging period (Figure 3.5).

Figure 3.5. Damage and feeding habits of *Sitophilus granarius* L.

3.1.2 Inert Dusts

3.1.2.1 Bentonite ((Na,Ca) (Al,Mg) 6(Si_4O_{10}) 3$(OH)_6$ nH_2O))

Bentonite is member of smectite group under classification of silicates. Texture of bentonite is rocky and highly colloidal with creamy-white color (Figure 3.6). This texture is coming from devitrification of volcanic ash and consistency from montmorillonite. Main characteristic of bentonite comes from its large quantity of water absorption, volume increase and plasticity. They are mainly seen in formation of younger rocks (Adamis et al., 2005).

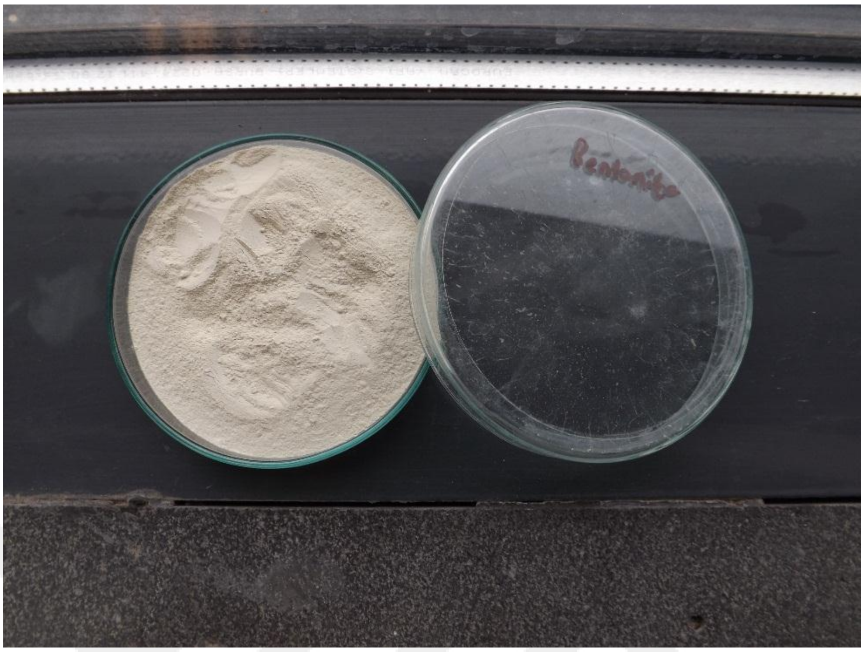

Figure 3.6. Bentonite material used in experiment

Due to its attractive properties, bentonite has been using in many industrial areas like projects in architecture and buildings, ceramics, cosmetics, medicines and so on. They can also be used in agricultural sector as an additive material for fertilizers and pesticides. Some of countries with high deposits of bentonite could use as refining material of petroleum as well (Grim and Güven, 1978). Turkey has also high deposits of bentonites in Ankara, Çankırı, Balıkesir and Ordu provinces which are maninly used in drilling, casting and paper industries (İpekoğlu et al., 1997).

3.1.2.2 Halloysite ($Al_2Si_2O_5$ $(OH)_4 . 2H_2O$)

Halloysite is a double layer mineral consisted from aluminum silicate, which was firstly described in 1826. They are generally derived from volcanic ash and rock contained soils in sub-trophic and humid regions (Massaro et al., 2017). Halloysite and kaolinite have the same chemical composition but main difference is coming from higher water content of halloysite. Morphologically, they can be found in many types. For instance, there are spherodical and tabular halloysites due to their formation time and texture (Figure 3.7) (Joussein et al., 2005).

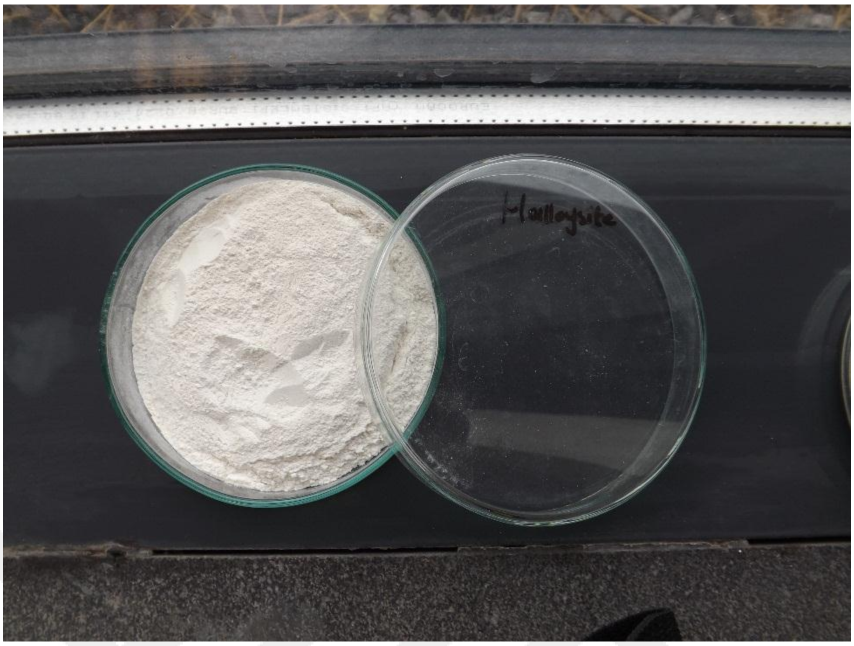

Figure 3.7. Halloysite material used in experiment

This texture give more advantages and possibilities to use in many important sectors especially in nanotechnology. Halloysite is used as nanocomposites, interlayering, absorbent for pollution and its remediation, it is also important for biosynthesis and encapsulation. This usage can vary in hydrated and dehydrated halloysite materials (Yuan et al., 2015).

Main production of halloysite is located in New Zealand. However, northwest deposits of Turkey (Çanakkale and Balıkesir provinces) has also high production of halloysite (Saklar et al., 2012).

3.1.2.3 Kaolin ($Al_4Si_4O_{10}$ $(OH)_8$)

The term kaolin is derived from a Chinese term "Kauling" referring name of a hill in China. It is the first place for kaolin mining. Therefore, kaolin is also called "China Clay" in many regions around the World (Prasad et al., 1991). Kaolin has whitish or creamy color mainly consisting from kaolinite crystals having pseudo-hexagonal shape with silica and alumina layer (Figure 3.8). This texture is formed by weathering of rocks, feldspars, granites and aluminum silicates (Murray, 2006).

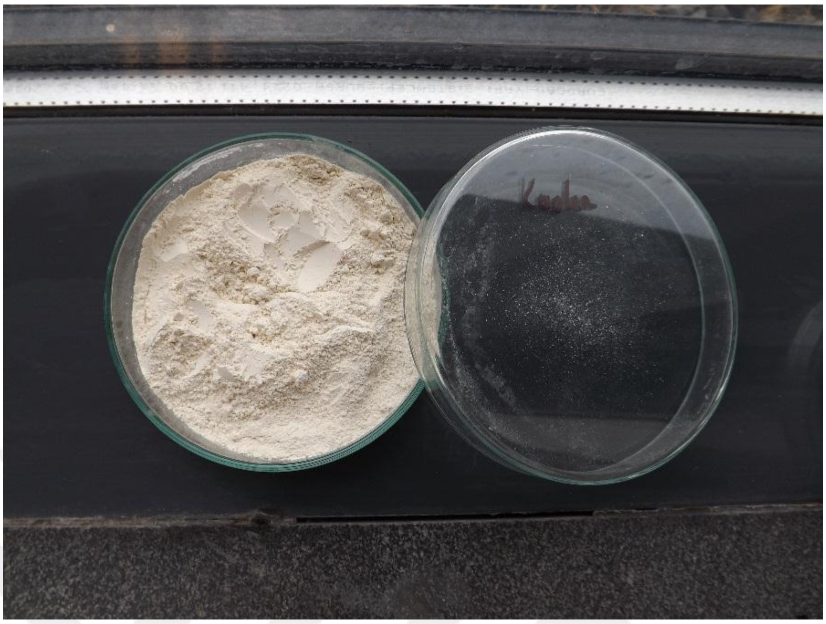

Figure 3.8. Kaolin material used in experiment

Kaolinite is main component of kaolin and provide less-conductivity of heat and electricity. Due to these characteristics and other specialties, kaolin is used in many industrial areas. For instances, paper, painting, ceramics, plastics, fiber glasses, cosmetics and fertilizer industries. It is also used in fruit, vegetable, cereal crop protection with its insecticidal effect (Prasad et al., 1991).

In Turkey, kaolin is mainly used in ceramics, paper and cement industry without any processing (DPT, 2001). However, there is also studies related to crop production and storage pest prevention including many insects that are harmful during pre-harvest and post-harvest periods. Usage of kaolin can vary due to its target and host plant physiology. It can be used directly or with particle film technology on fruits and vegetables (Nikpay, 2006; Yazıcı and Kaynak, 2007).

3.1.2.4 Nobleite (Calcium Borate Hydrate) ($CaB_6O_{10} . 4H_2O$)

Calcium borates are compounds that consisted from boric acid lime and slurry of aqua compounds having tendency for oxygen bonding. Nobleite is a member of this calcium borate group, which was discovered in California having white color (Figure 3.9). It is also analog of tunellite mineral having crystal texture with water insolubility. Although it can be mined in deposits, there are also processes for synthetic production (Schubert, 1997).

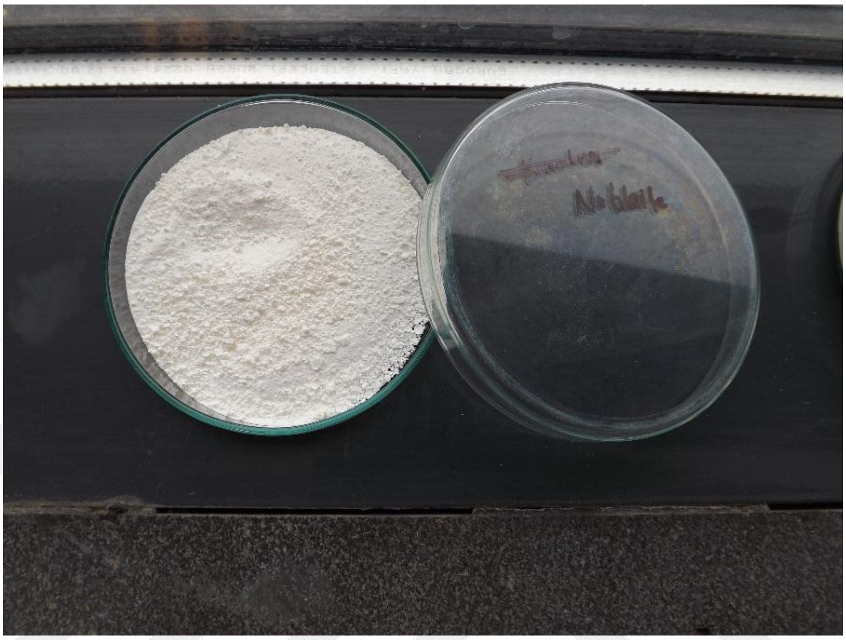

Figure 3.9. Nobleite material used in experiment

There are many usage sectors of calcium borates. Such as, glass, sanitation, dyeing, fire-retardant industries and so on. They were rearly used in agriculture sector (Durğun, 2010). Although there is not enough information about nobleite production, Turkey has high amount of boron deposits mainly in Eskişehir, Balıkesir and Kütahya provinces (Helvacı, 2003) which can have high potential for synthetic production.

3.1.2.5 Sepiolite ($Si_{12}O_{30}Mg_8(OH)_4 . (H2O)_4 . 8H_2O.$)

Sepiolite is grouped under hydrated magnesium silicates, which is structurally similar to palygorskite mineral except for its larger unit cells. It has 2:1 inverted structure with function on high capacity of absorption (H. H. Murray, 2006). The color of sepiolite is creamy and white but can vary due to its compactness and formation (Figure 3.10). Therefore, sepiolite in different regions may have different characteristics (Alvarez, 1984).

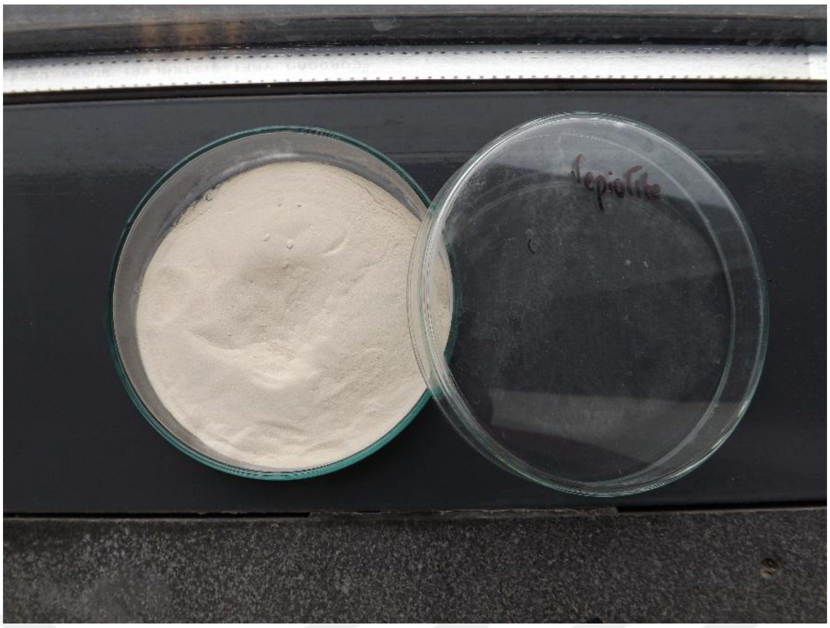

Figure 3.10. Sepiolite used in experiment

Although production of sepiolite is dominated by Spain, there are also some deposits in Turkey, especially in Eskişehir province. Usage of this material mainly divided into three sectors which are called as sorptive (pet-litter), catalytic transporters and rheological applications (include agriculture and construction industries) (Sabah and Çelik, 1999).

3.1.2.6 Zeolite (Li, Na, K) (Mg, Ca, Sr, Ba) $_d[Al_{(a+2d)}Si_{n-(a+2d)}O_{2n}]$. mH_2O)

Zeolites are originated from sedimentary and eruptive rocks which can be produced natural or synthetically having creamy and white color (Figure 3.11). General formula of zeolites can also vary due to variability of elements during formation. General formation have tetrahedral nodes by oxygen bridges (Gottardi and Galli, 1985).

Zeolites are mainly used against water pollution due to its ion exchange capacity, and in solar energy system as a heat exchanger. Zeolites also very common in agriculture and animal husbandry sectors. It is used as an additive material for fertilizers, poultry and livestock feeding (Zorbay and Arslan, 2012). There are also some laboratory studies for evaluation of insecticidal effect of zeolites as well.

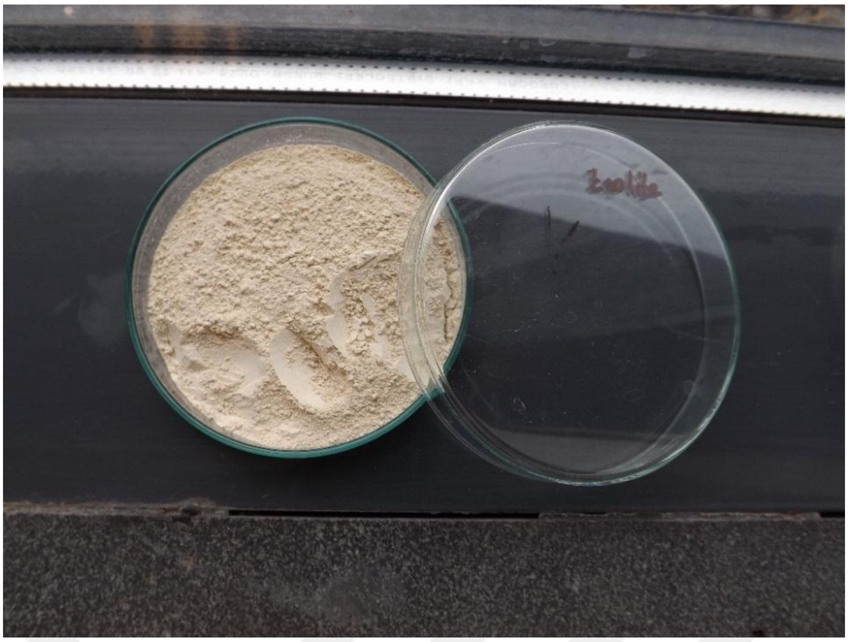

Figure 3.11. Zeolite used in experiment

Zeolite is mostly produced in China, Cuba, Japan and United States. Zeolite in Kütahya, Eskişehir, Kırşehir, Yozgat provinces are main production sources in Turkey (Soylu and Gökkuş, 2017).

3.1.2.7 Protector® and PyriSec®

Diatomaceous earth (DEs) are remains of fossil diatom organisms and include silica material. Deposits of DE have different characteristics due to their chemical formation and pH (Sağlam et al., 2017). Protector® and PyriSec® are commercial and modified DEs having creamy or whitish color, which are used against storage insect pests.

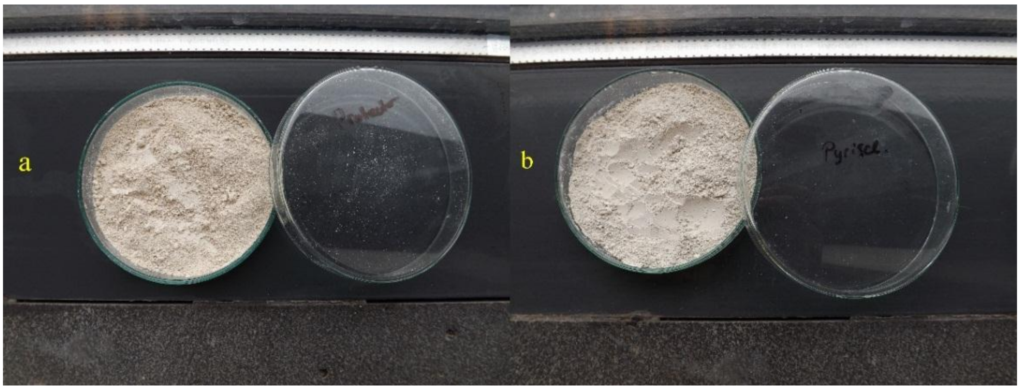

Figure 3.12. Protector® (a) and PyriSec® (b) used in experiment

Protector® is consisted of 69.7% of SiO_2, 5.89%, Al_2O_3, 0.414%, CaO and 1.05%, Fe_2O_3 (Intrachem, Italia) (Baldassari et al., 2008). Pyrisec® is consisted of 88% SiO_2 with 1.2% natural pyrethrum (25%) and 3.1% PbO (Biofa Gmhb, Germany) (Athanassiou and Kavallieratos, 2005). These two commercial DEs were used as standard in the experiments in this study (Figure 3.12).

3.2 Methods

3.2.1 Insect rearing and preparation of wheat grains

In this study, *S. granarius* adults were obtained from the stock culture at Niğde Ömer Halisdemir Univesity – Department of Plant Production and Technologies. Approximately 100 individuals were collected from the stock culture and transferred into a 1 liter glass jar with wheat grains (Figure 3.13). The wheat grains were washed and dried at 60 °C for 14 hours before using as a rearing media of insects. The insect culture were incubated at 25 ± 2 °C and 30%-40% RH during 72 hours in dark conditions for egg laying. Then, adult insects were removed and wheat grains were incubated at above-mentioned conditions to obtain single age cultures which were used in the experiments.

Figure 3.13. Incubation and rearing jars of *Sitophilus granarius* L.

3.2.2 Preparation of inert dusts

Inert dusts were provided from the Central Laboratory of Niğde Ömer Halisdemir University. They were collected from deposits in Turkey and grinded into fine powder form. The inert dusts were stored in Ziploc® bags at 25 ± 2 °C, in dark conditions. Inert dusts were also dried at 70 °C for 14 hours to remove moisture and any contaminants before they were used in each experiment (Figure 3.14).

Figure 3.14. Incubation of inert dusts

3.2.3 Single dose toxicity tests

In single dose toxicity test, all inert dusts were tested at 2000 ppm (w/w) dose to determine their toxic effect. Each of them was weighed on a weighing boat (Sigma-Aldrich) using a four digit electronic scale (Shimadzu®, TW423L, Japan). The inert dust was transferred into a 10 ml of glass vial (VWR®, 548-0090, 46mm x 22.5mm) containing 5 grams of wheat grains and shaken for 60 seconds (Figure 3.15). Same age 10 *S. granarius* individuals were collected from the stock culture and released into the glass vial. In the control group, the wheat grain was not treated with any inert dusts. The inert dust standards Protector® and Pyrisec® were used at manufacturer recommended doses (2000 ppm). The glass vials was covered by cheesecloth to prevent escape of insects. The insects were incubated at 25 °C ± 2, 30%-40% RH in dark conditions. Dead insects were counted and recorded at every 24 hours for 7 days. Randomized block design was used in experimental set up and the whole study was repeated 4 times. And block criteria was time (days) in this experiment.

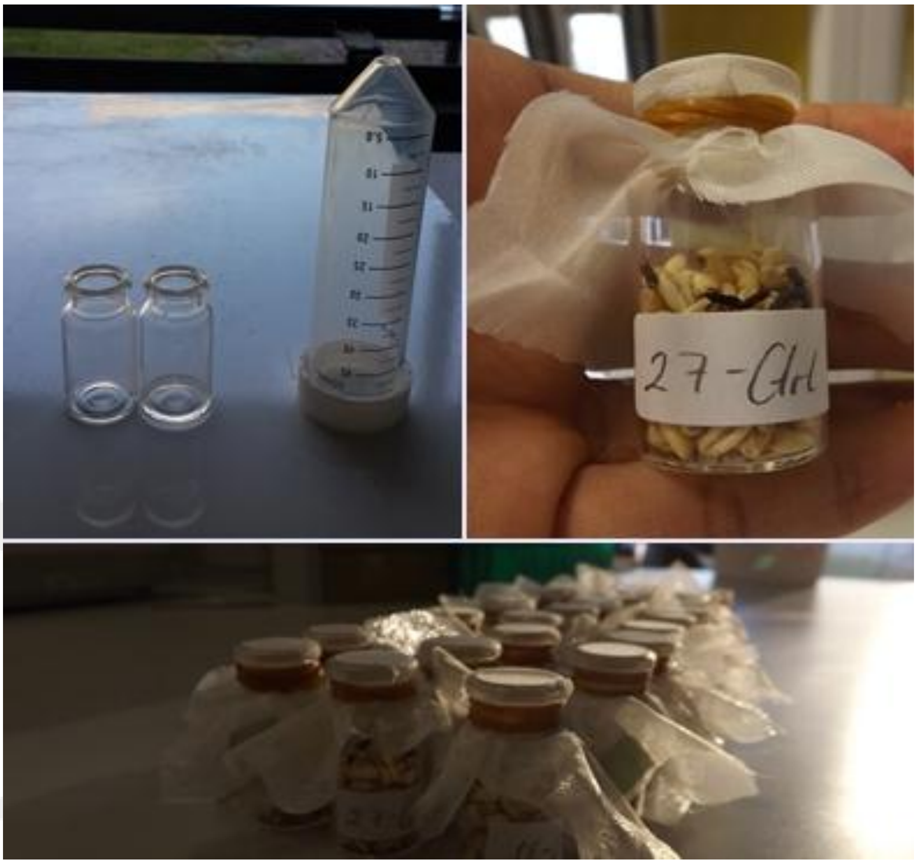

Figure 3.15. Preparation of samples for dose tests

3.2.4 Dose-response bioassay

In dose-response bioassay, the most toxic inert dusts, based on single dose toxicity bioassay, nobleite and kaolin were tested at 100, 500, 700, 1000, 1300 and 2000 ppm dose. The standards Protector® and PyriSec® were also tested at the same dose from 100 ppm to 2000 ppm.

The application of inert dusts were performed as described in single dose screening tests. The incubation conditions were the same as in previous experiment and the mortality of the granary weevil was checked at 24 hour intervals for 7 days. Experiment was set in randomized block design. Each block consisted of all doses of inert dusts and control group. The experiment was repeated at 4 different time as a block criteria. Total 720 insects were used for each inert dust except control in which 120 insects were used.

3.2.5 Lethal time

In the lethal time study, sub-lethal dose LC_{25}, based on dose-response bioassay was used with methodology modified from study of Mbata and Phillips (2001). The LC_{25} of an inert dust was weightened and applied to 5 gr of wheat kernels. This sub-lethal dose were selected to examine lethal time without effecting them via direct mortality. Ten the same age *S. granarius* adults were transferred into a vial and incubated at conditions described in single dose toxicity experiment conditions. In the control group, the insects were incubated with 5 gr sterile wheat kernel. After 24, 48, 72, 96, 120, 144, 168, 192 and 216 hours the dead adults were counted and recorded. The experiment was set up in randomized block desing and each block consisted of each time interval for every inert dusts. The whole experiment was repeated three times. Total 810 insects were used for each inert dust and also for the control group.

3.2.6 Antifeedant effect

Antifeedant effect of inert dusts were tested using LC_{10}, based on dose-response bioassay. Five grams of wheat grains was weighed and placed into a glass vials. LC_{10} of inert dust was added into these glass vials and then shaken during 60 seconds. Reason for applying LC_{10} is to see antifeedant effect on insects without effecting them via direct mortality. Same age 10 of *S. granarius* individuals were released into a glass vial. In control group, 5 gram of grains were directly transferred into a glass vial without inert dusts. Positive control samples were also prepared for minimizing errors, which directly affected from incubation conditions and grains physiology. Therefore, positive control samples had only grains without insects and inert dusts to optimize our applications according to these normal grain weight changes.

The insects were incubated at 25 ± 2 °C, 30%-40% RH and in dark conditions for 14 days. After this period, insects were removed; grains were weighed again and recorded. Randomized block experimental design was used and the experiment was repeated at 4 times. Block criteria was time (days) in this experiment. Antifeedant effect of treatments were calculated by using mentioned formula in Kutas et al. (2003);

$$AE(\%) = (1 - \frac{T}{C}) * 100 \qquad (3.1)$$

AE(%): Percentage of antifeedant effect

T: Weight loss in treated groups

C: Weight loss in control groups

3.2.7 Repellent effect

Repellent activity of inert dusts were tested at LC_{10}. Reason for applying LC_{10} is to see repellency effect on insects without effecting via mortality. This method were modified from study of Kamruzzaman et al., (2005). Approximately 5 gram of wheat grains were weighed via precise scale and placed into glass vials. Inert dusts were added into these glass vials and were shaken during 60 seconds. Then, inert dust applied and control grains were separately added in three portioned (Y type) petri dishes (90mm, LP Italiana, Italy). Last portion was used as release area of 10 same age insects. On the control group, both portion were consisted with control grains (Figure 3.16).

These samples were incubated at 25 ± 2 °C, 30%-40% RH and in dark conditions. After 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168, 192 and 216 hours, insects' choice was recorded. The experiment was set up in randomized block (in criteria of time) design and the whole experiment was repeated 4 times.

Figure 3.16. Experimental setup of repellent effect

Formula which were described in study of Licciardello et al. (2013) was modified by adding insect release area under black spaces of experiment setup. Number of insects that were on untreated grains and blank spaces (U) were divided by total number insects (10), and multiplied with 100 to calculate repellency index (RI%).

$$\text{RI (\%)} = \left(\frac{U}{10}\right) * 100 \qquad (3.2)$$

3.2.8 Antioviposition effect

Antioviposition effect of inert dusts was studied using sub-lethal (LC_{10}) doses for each material. Reason for applying LC_{10} is to see antioviposition effect of inert dusts without effecting insects via direct mortality. Five grams of wheat grains were weighed and placed into a glass vial. LC_{10} of an inert dust was added into the glass vial and were shaken during 60 seconds.

Figure 3.17. Emerged insects and grains in the experiment

Female and male granary weevils were differentiated through their rostrum thicknesses, size and checking with their angle of abdomen which was described in the study of Dinuta et al., (2009). Same age 10 of *S. granarius* (5 female, 5 male) were released into a glass vial and incubated (at 25 ± 2 °C, 30%-40% RH and in dark conditions) for 7 days. After 7 days, adults were removed and the wheat kernels were further incubated for 50 days at the same conditions in the Petri dishes (Figure 3.17).

After 50 days, emerged insects were recorded for each sample and inhibition rate (IR%) was calculated as described in study of Alkan et al., (2019). Randomized block (in criteria of time) design was used in experiment set up. The experiment was repeated 4 times.

$$IR\ (\%) = (C_n - T_n) / C_n * 100 \qquad (3.3)$$

C_n = Emerged insects in control samples
T_n = Emerged insects in inert dust applied samples

3.2.9 Statistical method and analysis

Single doses, antifeedant and repellency results were subjected to arcsine transformation and analyzed by multiple comparison test of Tukey HSD with ANOVA, using IBM SPSS® Statistics Software (22.0). Dose-response bioassay results were analyzed through probit analysis with POLO-PLUS Leora (1994) for LC_{50} and LC_{90} values with their upper and lower intervals.

Lethal time results were also analyzed using probit analysis with POLO-PLUS Leora (1994) programme. LT_{10}, LT_{50} and LT_{90} results were described with their lower and upper bound intervals.

Anti-ovipositon results were calculated using the formulas described in material method section. These results were transformed to arcsine and then analyzed with ANOVA test using IBM SPSS® Statistics Software (22.0) programme.

CHAPTER IV

RESULTS

4.1 Single dose test results

Single dose test results were presented in Table 4.1. The results show that, there were significant differences between treatments at all mentioned time intervals (F= 115.30 for 48h, F= 92.99 for 72h, F= 248.64 for 96h, F= 264.63 for 120h, F= 230.58 for 144h and F= 330.10 for 168h) (df= 8, 27 for all time intervals). Kaolin and inert dust standards (PyriSec® and Protector®) produced the greatest mortality with 53.48%, 68.00% and 65.77% mortality, after 48 hours respectively. While sepiolite caused 7.03% mortality, the mortality rate was 2.11% in nobleite treatment. The least mortality rate was observed in bentonite, halloysite, and zeolite inert dusts.

Table 4.1. Mortality effect of inert dusts (2000ppm) on *Sitophilus granarius* L at different time intervals

	Mortality ratio (%) ± SE** (%)					
Treatment	**48 hours**	**72 hours**	**96 hours**	**120 hours**	**144 hours**	**168 hours**
Bentonite	0.07±0.24c*	22.04±0.98c	45.57±0.58d	62.94±0.68c	74.21±0.58c	82.59±0.73b
Halloysite	0.07±0.24c	23.21±0.52c	40.71±0.24de	56.13±0.64c	67.06±0.38c	72.97±0.30bc
Nobleite	2.11±0.96bc	59.53±0.49b	95.59±1.06b	100.00±0.0a	100.00±0.0a	100.00±0.0a
Kaolin	53.48±0.52a	94.13±1.4a	99.85±0.51a	100.00±0.0a	100.00±0.0a	100.00±0.0a
Sepiolite	7.03±1.42b	55.13±0.57b	71.12±0.22c	87.89±1.17b	95.19±1.24b	99.85±0.51a
Zeolite	0.0±0.0c	15.5±1.3c	27.60±0.52e	48.28±0.26c	62.06±0.51c	68.55±0.17c
PyriSec®	68.00±0.48a	95.10±1.32a	100.00±0.0a	100.00±0.0a	100.00±0.0a	100.00±0.0a
Protector®	65.77±0.67a	96.27±1.19a	100.00±0.0a	100.00±0.0a	100.00±0.0a	100.00±0.0a
Contol	0.07±0.24c	0.07±0.24d	0.28±0.45f	0.28±0.45d	0.64±0.60d	0.64±0.60d

* Statistically, different letters next to SE values indicate significance level of each treatments in each column (Tukey HSD tests, P<0.05)
** SE: Standard Error

Mortality effect of the tested inert dusts were significantly different from the control group after 72 hours (F= 92.99, df= 8, 27). The sequence of mortality effects of inert dusts at 72 hours were parallel to 48 hours (F= 115.30, df= 8, 27) results. Kaolin, PyriSec® and Protector® caused 94.13%, 95.10% and 96.27% mortality, respectively.

While nobleite and sepiolite treatments produced 59.53% and 55.13% respectively. Similar to 48 hours, the least mortality effect were observed in bentonite, halloysite, and zeolite inert dusts.

After 96 hours ($F= 248.64$, $df= 8, 27$) incubation, all inert dusts incrased their sequence of effectiveness. However, the most dramatic increase was seen in nobleite and its toxicity nearly doubled in 24 hours. Kaoline, PyriSec® and Protector® killed all tested insects at this time interval. Zeolite, bentonite and halloysite also increased their effectiveness in parallel to the incubation time similar to sepiolite.

Nobleite, kaolin, PyriSec® and Protector® caused 100% mortality after 120 hours ($F= 264.63$, $df= 8, 27$) incubation. They were followed by sepiolite with 87.89% mortality rate. Bentonite, hallosite and zeolite produced 62.94, 56.13 and 48.28% mortality respectively. Sepiolite efficacy were between the most toxic group (nobloite, kaoline, PyriSec® and Protector®) and the least toxic group (bentonite, hallosite and zeolite) with 87.89% mortality.

After 144 and 168 hours ($F= 230.58$, $df= 8, 27$ and 330.10 $df= 8, 27$), sepiolite were achieved 95.19% and 99.85% mortality ratio. Zeolite, bentonite and halloysite also increased their sequence of effectiveness in parallel to the after 120 hours' results. Overall, the most promising mortality ratio were observed in kaolin and nobleite inert dusts.

4.2 Dose-response bioassay with inert dusts

Dose-reponse bioassays were carried out with nobleite, kaolin, PyriSec® and Protector® based on single dose screening bioassays results**.** PyriSec® appear to be the most toxic inert dust among the tested dusts with low LC values. Calculated LC_{10}, LC_{25}, LC_{50} and LC_{90} values for PyriSec® were significantly different from the values calculated for nobleite, and kaolin. Although these vaules for PyriSec® were less than the values calculated for Protector®**,** there was no significant differences between these two inert dust toxicity (Table 4.2).

LC_{10} values were 380, 360, 190 and 238 ppm for nobleite, kaolin, PyriSec® and Protector® respectively. LC_{25} values were 875, 618, 365 and 454 ppm, calculated LC_{50} values were 2208, 1132, 744 and 928 ppm for nobleite, kaolin, PyriSec**®,** Protector**®** respectively (Table 4.2). Interestingly, LC_{90} 95% confidence intervals of Protector**®** and kaolin overlapped and there was no significant difference between LC_{90} values of these two dusts. LC_{90} value for nobleite was 12788 ppm and it was the least toxic inert dust among the tested dusts. PyriSec® was the most toxic inert dust with 2892 ppm for LC_{90} value.

Table 4.2. Dose-response bioassay results of different inert dusts on *Sitophilus granarius* L. after 72 hours

			Lethal Dose (ppm)				
Treatment	**Slope ± SE**	**Intercept**	**LC_{10} (95% CI*)**	**LC_{25} (95% CI)**	**LC_{50} (95% CI)**	**LC_{90} (95% CI)**	**Chi-square**
Nobleite	1.68±0.22	-1.75	380 (264-484)	875 (722-1064)	2208 (1704-3288)	12788 (7118-34352)	3.46
Kaolin	2.56±0.27	-1.93	360 (270-435)	618 (522-702)	1132 (1016-1280)	3574 (2800-5122)	1.68
PyriSec®	2.17±0.19	-1.24	190 (138-242)	365 (295-432)	744 (638-870)	2892 (2240-4088)	2.96
Protector®	2.17±0.20	-1.45	238 (172-260)	454 (370-532)	928 (808-1070)	3604 (2780-5170)	2.44

*CI: Confidence interval

The reason for using 72 hours results is due to longer exposure time in lethal time were not provide accurate results in relatively higher LC amount and, insignificance were seen in longer exposure time.

4.3 Lethal time results

Calculated LT values for nobleite, kaolin, PyriSec® and Protector® were presented in Table 4.3. LT_{10} values were 76.63 and 66.19 hours for nobleite and kaolin. They were significantly longer than LT_{10} of PyriSec® and Protector® (56.36 and 58.50h). LT_{50} values were ranged form 111.07 hours to 169.64 hours. LT_{50} of PyriSec® and Protector® were significantly shorter than LT_{50} of nobleite and kaolin. The time took to reach 90% mortality was 375.51 hours for Nobleite while it was 334.75 hours in kaolin.

The least time required to produce 90% mortality was calculated for PyriSec® with 218.87 hours and it was followed by Protector® with 234.72 hours.

Table 4.3. Lethal time results of inert dusts at LC_{25} on *Sitophilus granarius* L.

			Lethal Time (h)			
Treatment	**Slope**	**Intercept**	**LT_{10}**	**LT_{50}**	**LT_{90}**	**Chi-Square**
Nobleite	3.71	-8.28	76.63 (66.71- 85.06)	169.64 (158.47- 183.70)	375.51 (321.70- 464.22)	2.55
Kaolin	3.64	-7.91	66.19 (57.13- 74.04)	148.86 (139.57- 159.67)	334.75 (291.41- 402.84)	3.26
PyriSec®	4.35	-8.89	56.36 (49.15- 62.76)	111.07 (104.37- 117.84)	218.87 (199.72- 245.51)	4.74
Protector®	4.24	-8.78	58.50 (51.31- 64.90)	117.18 (110.53- 123.96)	234.72 (214.12- 263.32)	1.53

4.4 Antifeedant effect of inert dusts

Antifeedant effects (AE%) of inert dusts against *S. granarius* are presented in Table 4.4. There was significant difference between treatments (F= 46.50, df= 3, 12). They were determined by using sub-lethal dose (LC_{10}) effect of inert dusts. The greatest antifeedant effect was observed in nobleite treatment with 81.49%. It was followed by Protector® with 76.18%. Intrestingly, PyriSec® showed moderate antifeedant effect with 65.19%. Nobleite showed the highest antifeedant effect with 81.49% while lowest was seen in kaolin with 43.16%.

Table 4.4. Antifeedant effects of inert dusts againts *Sitophilus granarius* L. at LC_{10}

Treatment	**Antifeedant Effect ± SE* (%)**
Nobleite	81.49±1.32a
Kaolin	43.16±1.69c
PyriSec®	65.19±1.44b
Protector®	76.18±1.61a

* Statistically, different letters next to SE values indicate significance level of each treatments in each column (Tukey HSD tests, P<0.05)
** SE: Standard Error

4.5 Repellent effect of inert dusts against *Sitophilus granarius* L.

Repellent effect of inert dusts was investigated by using LC_{10} sub-lethal dose. The results are presented in Table 4.5. The distribution of the granary weevils between treatment was not significantly different except 24 hours (F= 2.82, df= 4, 15) and 120 hours (F= 2.81, df= 4, 15). However even in this hours repellency index not mostly different from control group. At 24 hours, PyriSec® produced repellency with 72.50% and it was higher than value of kaolin. The other treatments were between these two treatments. At 120 hours, Protector® repellency index was 69.76% and it was small difference as compare to the control group.

Table 4.5. Repellency index (%) of inert dusts againts *Sitophilus granarius* L. at LC_{10}

	Repellency Index ± SE (%)				
Treatment	**Nobleite**	**Kaolin**	**PyriSec®**	**Protector®**	**Control**
1 hour	57.68±2.36	59.81±2.86	69.70±7.16	67.78±4.04	70.48±1.47
2 hours	58.59±4.83	50.96±3.18	56.96±2.58	63.59±1.25	62.75±1.21
8 hours	62.55±7.47	55.40±4.13	63.48±1.91	70.84±2.26	65.58±1.56
24 hours	60.42±2.76ab*	56.83±3.81b	72.50±1.94a	67.27±1.69ab	61.21±1.52ab
72 hours	58.45±0.7	55.22±1.66	64.64±1.74	68.07±4.62	61.12±1.65
120 hours	53.39±2.67ab	54.35±3.70ab	59.04±4.13ab	69.76±1.52a	51.80±1.92b
168 hours	62.15±2.58	60.39±1.81	63.63±2.20	61.85±1.29	60.35±1.62
216 hours	66.27±1.54	62.60±3.99	65.30±4.25	73.17±1.65	58.49±1.29

* Statistically, different letters next to SE values indicate significance level treatments in each row (Tukey HSD tests, P<0.05)
** SE: Standard Error

4.6 Antioviposition effect of inert dusts

Antioviposition effects of inert dusts at LC_{10} are presented in Table 4.6. There was no siginificant differences between treatments. (F: 0.105, df= 3, 12; P>0.05). All inert dusts used in experiment produced some level of antioviposition effects on the granary weevil.

Table 4.6. Inhibition rates of inert dusts on *Sitophilus granarius* L.

Treatment	Inhibition Rate (%) ± SE (%)
Nobleite	59.00±3.44
Kaolin	67.15±4.48
PyriSec®	60.08±6.61
Protector®	66.64±2.95

The greatest effect was seen in kaolin treatment with 67.15% rate. Protector® reduced the number of emerging adult at 2/3 fold. PyriSec® and nobleite also reduced the number of emerged adults around 60%. These results indicate that, inert dust treatment limits the oviposition of females with coating surface of grains. It is unlikely that the inert dusts have any effect on developing larvae. Therefore, the effect seen in this experiment mainly associated with coating effect of inert dusts.

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CHAPTER V

DISCUSSION

Toxic and behavioral effects of inert dusts against one of the important stored product pest, *S. granarius*, were evaluated under labaoratory conditions. The studies indicate that, some of the inert dusts have both toxic and behavioural effects on the target insect species. Main mode of action of inert dusts are coming from their moisture absorbance capacity (Ebeling, 1971). Therefore, inert dusts with higher de-hydration characteristics could be an effective tools against stored products pest like *Sitophilus* spp., which are thought to be very sensentive to de-hydreation (Liska et al., 2017).

Single dose screening tests show that, kaolin and nobleite caused the greatest mortality at 2000 ppm and the mortality rate increased to 100.00% after 120 hours. Their effects were similar to the standart inert dusts Protector**®** and Pyrisec**®**. The other inert dusts were not as effective as the above-mentioned dusts. Zeolite, halloysite and bentonite mortality rates were 48.28 %, 56.13% and 62.94%, respectively in the same exposure time. The similar results with kaolin were reported in other studies. Liska et al. (2017), reported that KaMin™ 80 product, (water washed kaolin clay) caused 41.8% mortality on *S. oryzae* at 500 ppm after 168 hours. Toxic effect of kaolin against othert stored product pests were also observed by El-Sayed et al. (2010) who tested kaolin at four different doses (1, 2, 4 and 8 g/ 100 g wheat ratio) on *S. oryzae*. The greatest mortality was observed at 8 g /100 g dose with 91.6% mortality after 72 hours.

There is limited studies on insecticidal effects of nobleite on stored product pests including *S. granarius*. Although nobleite caused nearly similar mortality to the standard dust formulation Protector®, PyriSec® and kaolin, it took more time. This time gap could be related with physical properties of nobleite because it does not aggregate between intersegments of insect cuticle. This could be reason for taking relatively longer time for achieving 100.00 % mortality ratio as compared with other inert dusts (Figure 5.1).

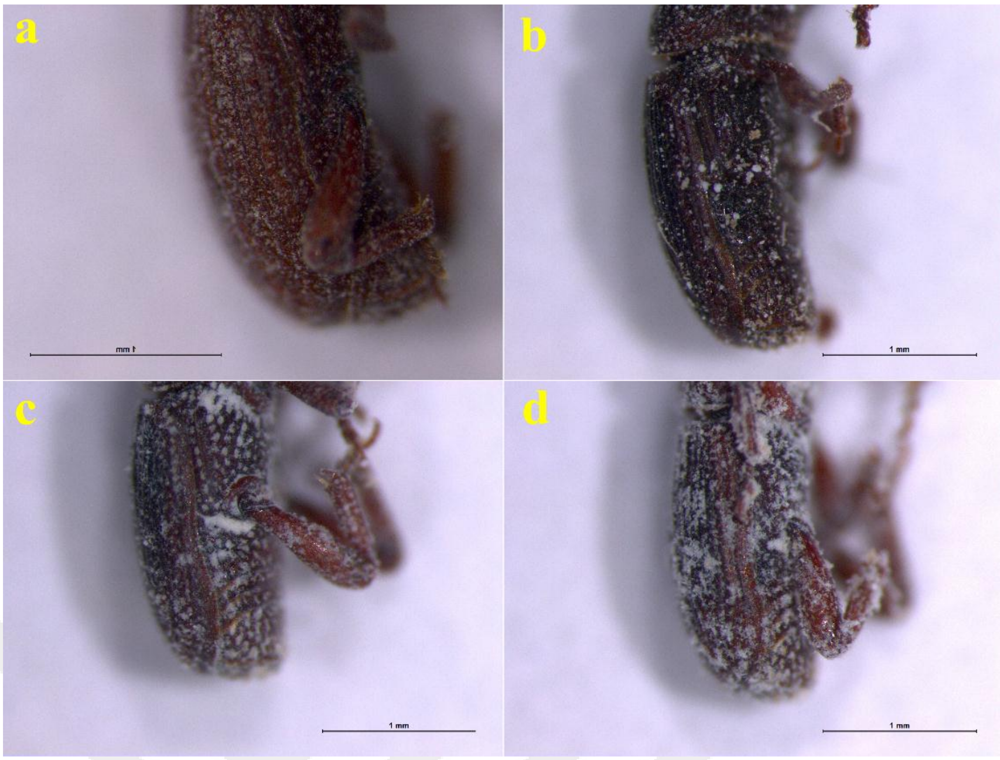

Figure 5.1. Density of inert dusts on intersegmental part of *Sitophilus granarius* abdomen at 2000 ppm: (a) kaolin, (b) nobleite, (c) Protector® and (d) PyriSec®

In the current study, bentonite caused moderate mortality to the granary weevil. The similar toxicity level of bentonite was reported by Liska et al. (2017). The mortality rate of *S. oryzae* was 27.1% at 500 ppm after 7 days. However, in the same study zeolite (Zeolite Slovakia 1, 2, and 3) produced 78.2% and 97.3% mortality on the rice weevil while zeolite Serbia caused 51.4% mortality. These results showed that, the source of inert dust have effects on their toxicity because geographical region have impact on inert dust characteristics and composition of particles. Kundu et al. (2018) studied bentonite insecticidal effect on the rice weevil at 10% (w/w) dose. He reported that, bentonite had low toxicity on the weevil with 19.88% mortality after 7 days. It is thought that low insecticidal activity of bentonite is coming from its lower desiccation capacity and particle form, which could have reduced its coating capacity to grains (Figure 5.3).

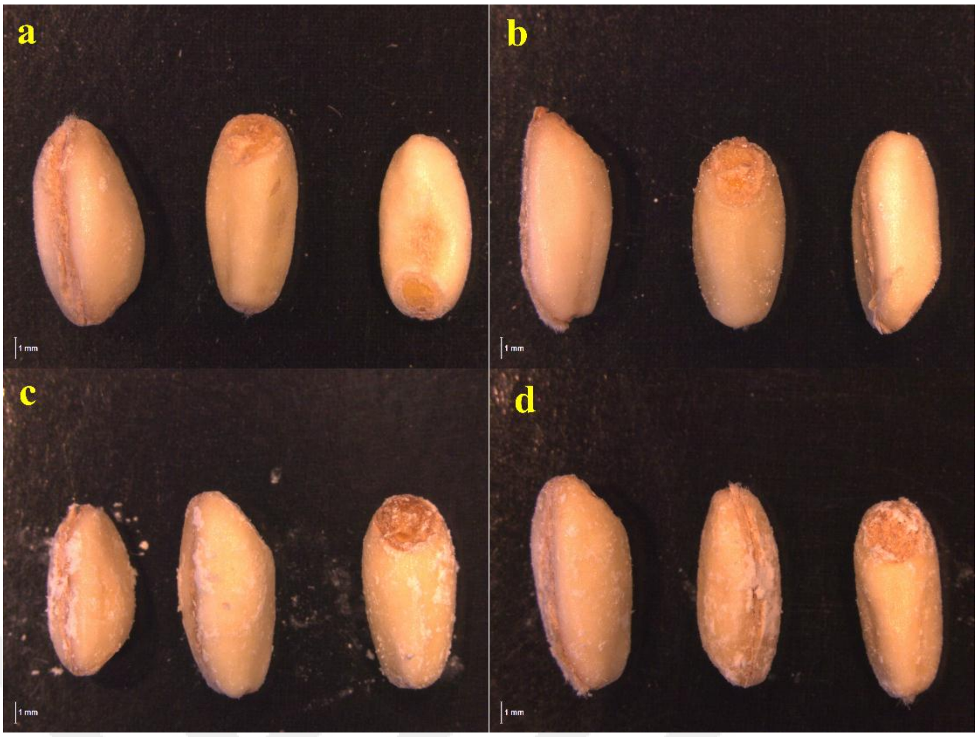

Figure 5.2. Coating and volume differences between inert dusts at 2000 ppm: (a) kaolin, (b) nobleite, (c) Protector® and (d) PyriSec®

Dose-response bioassays with kaolin and nobleite showed that, these two inert dusts have high mortality on *S. granarius*. Hussain Ahmad Mahdi and Khalequzzaman (2006) calculated 12078.15 ppm LC_{50} value for kaolin on *C. maculates* after 48 hours. It was calculated as 1132 ppm LC_{50} on *S. granarius* after 72 hours in this study. The experimental procedure as well as particle size, chemical composition and water content of kaolin with different insect species may cause the difference between calculated LC_{50} values. As explained above, the origin of inert dust has effects on their toxicity (Liska et al. 2017). The difference between their results with the current study result could be due to inert dusts' origin as explained above. Calculated LC_{50} value of kaolin is also comparable with the other studies in which DE materials were tested on *S. granarius* (El-Aziz and El-Ghany, 2018; Shams et al., 2011).

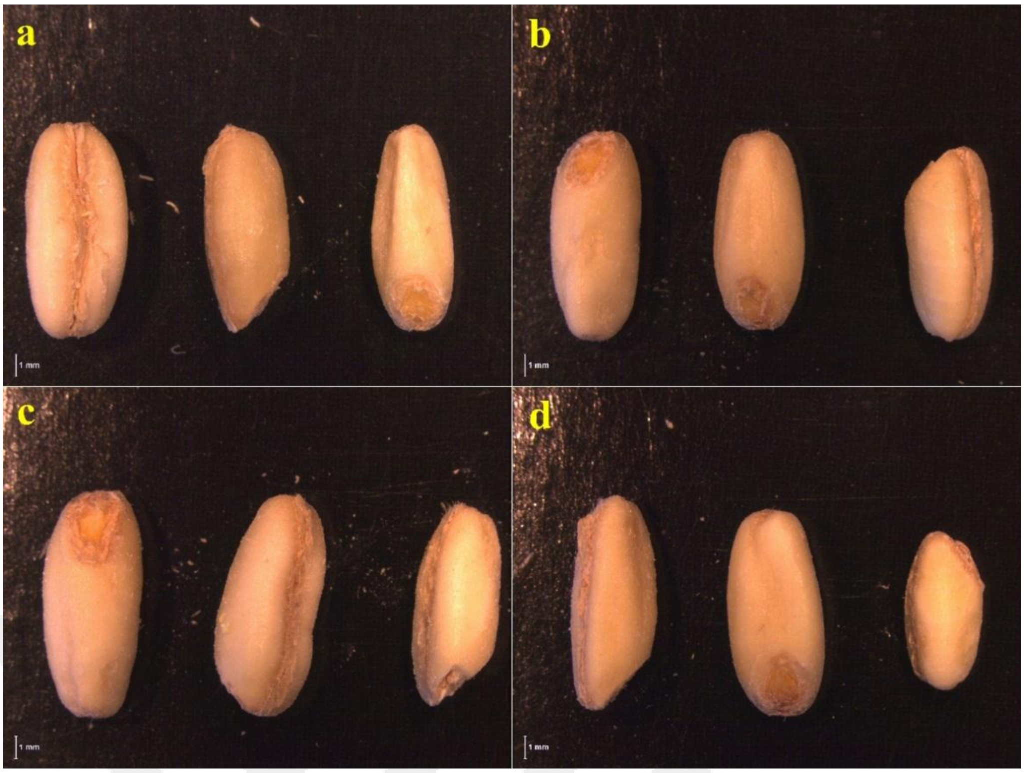

Figure 5.3. Coating and volume differences between inert dusts at 2000 ppm: (a) halloysite, (b) bentonite (c) sepiolite and (d) zeolite

Calculated LT_{90} was 375.51, 334.75, 218.87 and 234.72 hours for nobleite, kaolin, PyriSec® and Protector®, respectively. These results indicate that, kaolin requires less time to cause mortality on *S. granarius* than nobleite does. The similar phenomone was also observed in the single dose screening. The reason for requiring longer time to produce its effect are thought to be related with its physical characteristics (Figure 5.1). LT values were calculated using different inert dusts against various stored product insects. Stadler et al. (2012) tested nanostructured alumina inert material on *S. oryzae* at 250 ppm and calculated LT_{95} which was 261.6 hours. In the current study, the doses were 620 and 875 ppm for kaolin and nobleite, respectively. This result indicate that nanostructured alumina require less time compared with the tested inert dusts in the current study. However, the tested insect species are different and also, *S. granarius* is larger than *S. oryzae*, so the previous may require more amount of to get affected as compared to the latter one.

Antifeedant study of kaolin and nobleite show that, nobleite has a high potential to prevent feeding of *S. granarius* on wheat grains with 81.49% antifeedant effect. While kaolin caused the least antifeedant effect with 43.16%, the standart inert dusts Protector® and PyriSec® reduced feeding 65.19 % and 76.18%. The similar results were reported by other scientist Kundu et al. (2018), studied antifeedant effects of kaolin against *S. oryzae* and found that kaolin did not cause any significant reduction in feeding of the rice weevil.

Repellency effects of inert dusts study show that, differences among treatments were observed at 24 and 120 hours but mostly not different from control group. Overall, the inert dusts do not repel the adult granary weevils as they were present both in treated and untreated section of the petri dish. Although the inert dusts caused reduction in feeding of the insect, covering of cereal kernels with the dusts seem to not effectively enough to repelling insects. Repellency of many plant essential oils against stored product pests was reported and their effects are thought to be taste receptor mediated (Nawrot and Harmatha, 2012). It seems that the inert dust do not cause any stimulation on chemoreceptors of the granary weevil.

Antioviposition effects of any componds especially against stored product insects are crucial properties in reducing the infestation rate especially in big storage houses. All tested inert dusts have antiovipositional effect on *S. granarius* female. The calculated rates were 59.00% 67.15% 60.08% and 66.64% for nobleite, kaolin, PyriSec® and Protector® respectively. These results indicate that coating of inert dusts to the kernels prevents female egg laying (Figure 5.2). Kaolin produced higher inhibition rate than nobleite does. Similar antioviposition effects of kaolin on other *Sitophilus* species was reported. Liska et al. (2017) studied antiovipositon effects of KaMin™ 80 product, (water washed kaolin clay) on *S. oryzae* at 500 ppm. It was found that, the treatment caused 49.2 % reduction in egg laying. This study indicate that inert dusts have antioviposition effect potential and it should be further tested at different doses against various stored product insects.

CHAPTER VI

CONCLUSION AND FUTURE ASPECTS

In the current study, some of inert dusts' insecticidal propreties were evaluated under laboratory conditions. Dose test results showed that, kaolin and nobleite have some potential in the control of *S. granarius* population and the dose needed to produce the high efficacy was relatively lower for kaolin material. Kaolin was also effective to produce high percentage motality in shorter period of time comparaed with the other inert dust, nobleite. However, the antifeedant effect of nobleite was more pronounced than kaolin but further tests with nobleite at different doses against various stored product insects are needed to discover full potential of the dust. Both inert dusts have anti-ovipositional effect and, reduced the offspring of *S. granarius*. These results have some potential because they indicate that the inert dusts limit infestations as well as population increase in storage houses.

Kaolin and nobleite appear to have both toxic and behavioural effects on the granary weevils. They could have potential to get developed as commercial products against the granary weevil. However, further studies with these inert dusts against the granary weevil and also other stored product insect pests especially in different cereal grains as well as at different temperature and humidities could help to explore the full potential of these inert dusts as control tools. Therefore, future studies should concentrate on these missing parts that are not tested in the current study.

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