# A Preliminary Study of Use Some Inert Dusts Against Cowpea Seed Beetle *Callosobruchus maculatus* (F.) under Laboratory Conditions

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**Abstract**

The study aimed to test the effectiveness of five different inert dusts (silica, kaolinite, pumice stone, salt rock, zeolite) against the *Callosobruchus maculatus* (F.) (Coleoptera, Bruchidae) at a concentration of 5g/kg cowpea seeds. The study was carried out in incubator with fixed temperature (27±1 °C) and humidity (60±5%) at Biotechnology Research Center, Al-Baath University, Homs, Syria. The results showed that all inert dusts tested were effective against *C. maculatus* and the highest mortality ratio was observed in kaolinite treatments (94.86, 94.72, 85.88) % after 24, 48 and 72 hours respectively, but the lowest mortality ratio was observed in salt rock treatments (48.00, 31.43 and 26.27) %. Kaolinite was the best in its effectiveness on the reduction of new adult emergence (99.29%), weight loss (99.01%) and damage ratio (99.39%) in comparison with other inert dusts tested.

**Keywords**: Inert dusts, Cowpea seed beetle, *Callosobruchus maculatus*.

## Introduction:

Growing awareness and global attention resulting from the myriad problems with the use of chemical pesticides that have led to the development of safe strategies for the environment and human health. In particular, the frequent use of these pesticides revealed several problems that were not taken into account, as some of the pesticides used had a wide spectrum and were highly toxic to a large number of insect species, this has led to the killing of parasites and predators (vital enemies), weakening their role in the natural control process, causing serious environmental imbalance, in addition to their expensive cost, and causing significant damage to non-target organisms such as pets, birds, bees and humans (Akunne and Okonkwo, 2006; Akinneye and Ogungbite, 2013; Kpoviessi *et al.*, 2017).

Hence the search for relatively safe alternatives to these pesticides, such as inert dusts, furnace ashes, extracts, plant oils and others. It affects insects and delays the development of resistance, is less toxic to mammals and can be easily produced and used (Ketia *et al.*, 2001; Upadhyay and Ahmad, 2011; Rajasri *et al.*, 2014; Ahmady *et al.*, 2017).

Legume beetles, of the order Coleoptera family Bruchidae, are among the most important pests that attack legumes. The cowpea seed beetle, *Callosobruchus maculatus* (F.), is one of the most important species. The average adult life is between 9-12 days (Devi and Devi, 2014).
The damage is very large and is caused by the larvae that feeds on the seed contents from the inside and do not leave them before turning into adult, and the seeds infected with the cowpea beetle lose a large part of their content, which negatively affects the germination, and reduces the nutritional and marketing value of the seeds (Zannou *et al*., 2003).
The weight loss of seeds stored in India as a result of infection with the cowpea beetle was estimated at 55-60% and the decrease in the protein content of seeds was estimated at 45.5-66%, while the percentage of loss in cowpea stores in Nigeria as a result of infection with the cowpea beetle was 60% within three months of storage (Wakil *et al*., 2010). In Zimbabwe, the cowpea beetle was found to cause more than 90% loss in cowpea seed weight after 6 months of storage (Seck *et al*., 1991), and sometimes 100% of the stored seeds were damaged (Shams *et al*., 2011). Inert dusts are used in many industrial and agricultural fields, and can be used as insecticides to control pests in stored products. Its use is considered one of the control methods, as it is characterized by its cheap price, ease of application, and protection for grains stored for a long time, including powders kaolinite (Korunic *et al*., 1996; Fields and Korunic, 2002; Vojoudi *et al*., 2014). Including also silica, diatomaceous earth, which is the basis of its composition, sand, zeolite and clay, and its effectiveness is due to making the body of the insect lose water as a result of scratching the cuticle layer and absorbing water and fats, which causes hardening of the body and death, Kaolinite also affects the respiratory system by closing the respiratory holes, causing suffocation and death (Mahdi and Khalequzzaman, 2006; Upadhyay and Ahmad, 2011; Sokker *et al.,* 2012; Mohd and Akhtar, 2014; Kpoviessi *et al*., 2017).
The effectiveness of inert dusts also depends on some physiochemical properties such as molecular size, surface area, porosity, amount of absorption, grain type, type and phase of the insect, density of the insect community, and surrounding environmental conditions such as temperature and relative humidity in addition to the influence of other factors such as the size of the insect, and the rate of feeding of the insect, the thickness of the fat layer, the rate of water absorption from the internal guts, and it is possible that these factors are included in the difference in the proportion of death in different transactions (Frank; 2002; Mahmoud *et al*., 2010; Chelav *et al.*, 2013; Jianhua *et al*., 2017).
Mahmoud *et al*., (2010) treated the adult beetles of the cowpea seed beetle *C. maculatus* and the Chinese beetle *Callosobruchus chinensis* (L.) with Kaolinite powder, and the mortality rate in adults were 100% in both types of beetles with concentrations ranged from 0.2-1% w/w and testing the effectiveness of these concentrations after 1,2 and 3 months the number of adults emerging for the new generation and weight loss was 0% for both types of beetles at the previous concentrations at the first and the second months of the treatment of the seeds with Kaolinite powder. The number of adults emerging and weight loss in the third month was 0% when treated only at 0.8 and 1% concentrations for both types of beetles. Chelav *et al.*, (2013) found a decrease in the number of adults emerging compared to the control when cowpea beetle was treated with one of the diatom soil preparations. Diatom soil used against *Tribolium castaneum* and *Tribolium confusum* beetles, and the results showed a higher mortality rate in larvae compared to the control, with the *T. confusum* beetle being more affected by the treated compared with *T. castaneum* beetle and reducing in the percentage of the adult

emergence of both species (Sabbour and Abd Elaziz, 2015). Mixing dry seeds with diatom soil is a good way to protect products stored and therefore can be used as an alternative to conventional chemical pesticides and have no lasting effect on grains and their long-term effect (Vayias and Athanassiou, 2004; Wakil *et al*., 2010). Synthetic Zeolite powder is valued against 7 types of stored insects was mentioned of them cowpea seed beetle. The mortality rate of this insect was 100% after 72 hours of treatment and there was a decrease in adult emergence compared to the control (Subramanyam and Sehgal, 2014).

Some studies recommend the use of Kaolinite, sand, wood ashes, straw ashes and clay as effective substances for the control of warehouse insects and protect production in different countries of the world (David and Bhadriraju, 2000).

So, the study aims to test the effectiveness of five different inert dusts: silica, Kaolinite, pumice stone, salt rock, Zeolite against the *Callosobruchus maculatus* (F.) with fixed temperature 27±1 $\overset{\circ}{C}$ and humidity 60±5%.

**Materials and Methods:**

The adults of the cowpea seed beetle were collected from infected cowpea seeds, the adults were identified depending on their apparent form (Devi and Devi, 2014; Cabi, 2018). These adults were taken to create a permanent amount of breeding of the insect at a laboratory, by placing healthy seeds samples in transparent plastic cans of each liter capacity filled with healthy cowpea seeds in half, and seeds infected with cowpea beetle were added to them and the cans were covered with a muslin cloth fastened with rubber bands. The insects were left to reproduce in order to obtain as many adult insects as possible, and then the newly emerged insects were collected and placed in new plastic containers with intact seeds in order to expand breeding. The process continued until abundant numbers of homogenous adults of age and size were obtained for the use in the experiments.

The seeds were obtained from the municipal variety of Al-Marana, which belongs to the Talukh area of Homs, for the 2016 harvest season, the seeds were dried in the shade and purified from impurities and criminality, then the seeds were placed in a refrigerator at a temperature of -20° C and for 72 hours in order to get rid of all insect phases, if any. They were kept in the fridge at a temperature of 4 ° C until they were used in the experiments.

Kaolinite $Al_4(Si_4O_{10})(OH)_8$ was obtained from the Al- Mukhram area of Homs, silica $SiO_2$ from the Marmaleh white area of Homs, and Zeolite $[Si_3Al_2)O_{10})]^2[Si_4Al_2)O_{12}]^2$ from Al-Sis mountain of Damascus countryside. The required quantities of Kaolinite and silica were taken from the general institution of geology, and the quantities of Zeolite were taken from the general commission for scientific agricultural research (GCSAR). Pumice stone (composed of elements of aluminum, potassium, sodium and a small percentage of calcium, magnesium and iron) from the general phosphate company, and rock salt (NaCl) from the consumer public corporation. Previous materials were ground, smoothed and sifted using a 50 micron sieve to obtain homogeneity of the powder granules for use in subsequent experiments.

The concentration was 5 g/kg (based on preliminary experiments) for each type of powder studied, the moisture of the seeds was estimated using a drying oven at 130 ° C and for 2 hours at the beginning of the experiment reached 11.15%, then the weights were adjusted to a value of 14% humidity.

All experiments were carried out in an incubator at a constant temperature of 27±1 ° C and a constant relative humidity of 60± 5%, at the Biology Laboratory of the Biotechnology Research Center at the

University of Baath, Homs, Syria during 2017. By placing 70g cowpea seeds in a transparent bag to which the required powder was added by the concentration used, an amount of air was added to create a vacuum within the bag (for the purpose of stirring the contents of the bag so as to ensure a homogeneous coverage of the amount of powder used) then stir the bag for several minutes to ensure that the powder was distributed evenly over the seeds.

Seeds treated were distributed on repeaters by placing 10g powder-treated cowpea seeds per 250 $cm^3$ plastic cup (representing each duplicate cup). The process was repeated for all types of powders studied, while non-powder-treated seeds were placed within the control repeaters. Infection was carried out by placing 20 adults of the newly emerging common cowpea beetle in a sexual ratio of 1:1 in each repeated, covering the cups with muslin and incubating the specified heat and relative humidity. Transactions were followed up until the exit of the new generation and the following reading were taken:

1. The cumulative mortality ratio of adults after 24, 48 and 72 hours of treatment, then calculated the effectiveness in increasing the mortality ratio by applying the equation:

Efficacy = [(treatment mortality ratio - control mortality ratio)/treatment mortality ratio] × 100

2. Loss of seed weight ratio: This reading was taken after the death of all the adult offspring resulting from the different treatments and the control, the rates of loss were calculated based on the equation of Odeyemi and Daramola, (2000):

Loss of seed weight ratio = [(primitive weight-final weight)/(primitive weight)]×100

The effectiveness of the powders in reducing seed weight loss was then calculated by the formula

Efficacy= [(weight loss ratio by control - weight loss ratio by treatment)/ weight loss ratio by control)]×100

Noting the measurement of seed moisture in each repeater and then adjusting the weights to a value of 14% humidity.

3. Damage ratio of seeds: this reading was taken after the death of all adult offspring

This ratio is calculated by applying the equation:

Damage ratio= (number of punctured seeds/total seed number)×100

Then calculated the effectiveness of the powders in reducing the damage ratio by the formula

Effectiveness= [(damage ratio by control – damage ratio by treatment)/(damage ratio by control)]×100

4. The effectiveness of powders in reducing the adult emergence 4

The adult emergence was counted in the control and the various treatment, and then the effectiveness of the powders in reducing the adult emergence was calculated using an equation of Tapondju *et al*., (2002).

Efficacy =[( number of adult emergence by control – number of adult emergence by treatment) /(number of adult emergence by control)] ×100

**Statistical analysis:**

The experiment was designed according to the Complete Random Design (CRD), the results were statistically analyzed using Fisher F test and the averages were compared according to a test of LSD at the moral level of 0.01 using the program (Costat, 2008).

**Results:**

**1.The effectiveness of the tested inert dusts in increasing the average cumulative mortality ratio of adult's cowpea seed beetle:**

The results in (Table 1) show the effect of the inert dusts studied in increasing the average percentage of cumulative mortality ratio of adult's cowpea beetle within each time. The kaolinite powder achieved the highest impact on the cowpea beetle by a significant difference over the powders of silica, pumice stone and rock salt. the average efficacy values in increasing of the cumulative mortality ratio of adult's cowpea beetle were 94.86, 66.0, 64.0 and 48.0% respectively after 24 hours, while apparent differences between kaolinite and zeolite were recorded. After the time of 48 and 72 h on the experiment, kaolinite powder also surpasses all other powders studied and by a significant difference where the effectiveness of Kaolinite increased the average cumulative mortality ratio of adults to 94.72 and 85.88%, respectively. No significant differences were recorded between zeolite and silica treatment after 48 h, with the average efficacy in increasing the cumulative mortality ratio of adults 87.66 and 76.0%, respectively. The differences between silica, pumice stone and rock salt were significant, with efficacy reaching 76.00, 60.35 and 31.43%, respectively, after 48 h. Averages of efficacy values varied in increasing the cumulative mortality ratio of adults after 72 h of its treatment with the studied powders and the differences between these averages were significant, except for the treatment of silica and pumice stone. The average effectiveness in increasing the cumulative mortality ratio of adults in silica, pumice stone, rock salt and zeolite was 65.12, 59.05, 26.27 and 75.61%, respectively. The order of inert dusts was effective in increasing the cumulative mortality ratio of adults starting with the best:
kaolinite > zeolite > silica > pumice stone > rock salt

**Table 1. Effectiveness on the increase of adult's mortality ratio of the cowpea seed beetle**

| Powder type | Effectiveness on the increase of adult's mortality ratio% | | |
|---|---|---|---|
| | 24h | 48h | 72h |
| Silica | 66.00[b] | 76.00[b] | 65.12[c] |
| Kaolinite | 94.86[a] | 94.72[a] | 85.88[a] |
| Pumice stone | 64.00[b] | 60.35[c] | 59.05[c] |
| Rock salt | 48.00[c] | 31.43[d] | 26.27[d] |
| Zeolite | 89.04[a] | 87.66[b] | 75.61[b] |
| LSD $_{0.01}$ | **15.32** | **13.01** | **10.02** |

**Means followed by the same letters in each column are not significantly different at P=0.01.**

**2.The effectiveness of the tested inert dusts in reducing the adult emergence weight loss, and damage ratio:**

The results in Table (2) show the effectiveness of the inert dusts used in reducing the average percentage of the adult emergence, weight loss and damage ratio. Kaolinite achieved the best results compared to the other tested powders in terms of the three criteria studied. The values had significant differences in effect between Kaolinite and other powders for the average effectiveness of reducing the percentage of the adult emergence, weight loss and damage, giving the following values respectively: 99.29, 99.01 and 99.39%. There were no significant differences in effectiveness between the two silica and zeolite powders in terms of the three criteria studied, but they were apparent differences. the average percentage reduction values of the adult emergence were 85.78 and 78.16%, respectively, the average percentage reduction of weight loss was 80.02 and 71.48%, respectively, and the average percentage reduction of damage were 85.66 and 77.93%, respectively.

**Table 2. The effectiveness of the inert dusts studied in reducing the average percentage of the adult emergence, weight loss, and damage ratio**

| Powder type | Effectiveness of the adult emergence reduction | effectiveness of weight loss reduction | effectiveness of the damage ratio reduction |
|---|---|---|---|
| **Silica** | 85.78[b] | 80.02[b] | 85.66[b] |
| **Kaolinite** | 99.29[a] | 99.01[a] | 99.39[a] |
| **Pumice stone** | 68.14[c] | 60.98[c] | 65.44[c] |
| **Rock salt** | 61.72[c] | 53.83[c] | 62.17[c] |
| **Zeolite** | 78.16b | 71.48[b] | 77.93[b] |
| **LSD $_{0.01}$** | **8.84** | **10.38** | **8.42** |

**Means followed by the same letters in each column are not significantly different at P=0.01.**

On the other hand, pumice stone and rock salt achieved the lowest effectiveness compared to the other powders tested, in terms of the three criteria studied, and the average values of these powders had significant differences compared to those of the same criteria for other types of powders (Table, 2). On the other hand, the average values of both pumice stone and rock salt did not have significant differences between them.

The average percentage reduction of the adult emergence of pumice stone powder and rock salt were 68.14 and 61.72% respectively, the effectiveness of weight loss reduction were 60.98 and 53.83%, respectively, and damage ratio reduction were 65.44 and 62.17%.

The order of the types of powders in reducing the average percentage of the number adult emergence, weight loss and damage ratio starting from the best was:

kaolinite > silica > zeolite > pumice stone > rock salt

**Discussion:**

Kaolinite has the highest effect on the adults of cowpea seed beetle *C. maculatus*, because it has given the highest efficacy values in increasing the cumulative mortality ratio of adult's cowpea seed beetle during the times studied. In general, most of the powders tested showed efficacy in increasing the cumulative mortality ratio of the beetle above 50%, and the lowest efficacy values were recorded when treated with rock salt powder and at all times of the experience. In general, most of these powders have had an effect on the cowpea seed beetle, and this effect may be due to the fact that the insect body loses large amounts of water as a result of scratching the cuticle layer, powders absorb water, removing the fat layer, causing the body to harden and die. The high efficacy of Kaolinite may be due to its oily feel (Saeed, 2009), which makes it adhere well to the insect's body, covering a large area of the body wall, increasing the friction of the molecules of the Kaolinite and thus increasing the severity of the abrasion, which in turn leads to the loss of a large amount of body water faster compared to the other powders tested, in addition to the effect of Kaolinite on the respiratory system by closing the stomata, causing suffocation and death (Upadhyay and Ahmad, 2011; Mohd and Akhtar, 2014; Kpoviessi *et al*., 2017).

The decrease in the effectiveness of powders with the increased exposure time may be attributed to higher mortality ratio in control with increasing time because the average age of adults is between 9-12 days, this is explained by Devi & Devi, (2014). The effectiveness of most of these powders may be due to the reduction of the adult emergence, weight loss and damage ratio, due to the effective effect of these powders, especially Kaolinite powder on the cowpea beetle, and cause rapid death of the insect, and this leads to not giving it a longer life to lay eggs, as well as impeding the movement of adults between the mass of seeds treated, therefore, only reaches a limited number of seeds for laying eggs, and hindering the process of adhesion of eggs to the surface of the seeds, all of which positively reflects on reducing adult emergence, weight loss and damage ratio (Seck *et al*., 1991; Horng *et al*., 1999; Mahmoud *et al*., 2010; Shams *et al*., 2011).

**Conclusion:**

It could be concluded from the above that some inert dusts have proven effective in controlling cowpea beetle. The effect of the tested powders (kaolinite, silica, zeolite, pumice stone and rock salt) also varied on cowpea beetle, and the effectiveness of kaolinite, silica and zeolite powders were superior in increasing the cumulative mortality ratio, reducing the number of adults emergence, weight loss and damage ratio, compared to pumice stone and rock salt powders.

Also, it could be concluded that these results are encouraging and can be developed to benefit of them from the practical side towards the use of some of these inert dusts at the level of warehouses and stores in order to protect stored seeds from infection with cowpea seed beetle.

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