

Impact of kaolin-based particle film dusts on *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) after different storage periods of treated broad bean seeds

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Abstract

Adults of *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) were exposed to broad bean seeds treated with kaolin-based particle film dusts (powder) at different concentrations 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025% w/w and untreated control. The effect of kaolin powder film was clearly effective in the first month of storage period of the treated seeds resulted a 100% protection of treated seeds at high concentrations from 1.0 to 0.2 w/w for both tested insects. After three months of storage of the treated seeds only the highest two concentrations 1.0 and 0.8 w/w gave a 100% protection for both tested insects. After six months of storage of the treated seeds, kaolin powder still could protect the broad bean seeds against *C. maculatus* and *C. chinensis* attacks although the efficacy of kaolin powder decreased with aging. Thus, residual effect of Kaolin powder film was reduced by prolongation of the storage period. A negative relationship was recorded between the kaolin concentration and the tested biological parameters (number of eggs laid, hatchability, developmental period, F1 adult emergence, and seeds weight loss%) for both tested insects *C. maculatus* and *C. chinensis*. The comparison of the kaolin application methods, kaolin powder was more effective than kaolin suspension which gave a better protection to the treated seeds. Broad bean seeds viability was slightly affected by kaolin powder application, the reduction of germination was most greatest at highest concentration.

Keywords: Kaolin powder, Broad bean seeds, Protection, *Callosobruchus maculatus* (F.), *C. chinensis* (F.).

1. Introduction

Pulses such as broad beans, *Vicia faba* (L.) are important sources of proteins, fats, carbohydrates, sugars, vitamin B and minerals, which is the main component in the diet of many people (Aslam et al., 2006). It is attacked by several insect pests including Pulse Beetle, *Callosobruchus chinensis* (L.) and cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Both insects are considered serious pests (Ahmed et al., 2003) and cause immense damage every year to legume seeds. They attack legume seeds during the warm season and are able to generate exceedingly high levels of infestation within one or two generations on the host when conditions are acceptable (Shomar, 1963). They cause substantial quantitative and qualitative losses resulting in reductions in weight, market value and viability of seeds (Seck et al., 1996; Sekou et al., 2001).

As a result of insects' ability to rapidly increase in numbers, concerns exist over their ability to overcome the effects of contact insecticides applied to the stored legumes that are infested (Lorini, 2003). The choice of insecticides for pest control in stored legumes is limited due to food safety requirements imposed by national regulatory bodies within nations (Padin et al., 2002). Therefore this has led pest control researchers to examine alternative methods including the reappraisal of inert dusts.

The use of inert dust applied alone or in combination with other control methods is considered as a strong alternative method to the use of chemicals (Lorini et al., 2003) in protecting grains in many countries (e.g. Egypt, India and Mexico). Golob (1997) categorized inert dusts into five types which can be differentiated by their chemical composition or by their level of activity. Insect mortality is induced primarily as a result of desiccation: body water loss as a consequence of cuticle disruption by removal of epicuticular waxes (Alexander et al., 1944; Wigglesworth, 1944; Lorini et al., 2003). It has been postulated that the action of these materials is not dependent on metabolic pathways and therefore resistance should not occur (Ebling, 1971). In addition, inert dusts exhibit low mammalian toxicity of these materials (e.g. insect acute oral rate LD50 >5000 mg/kg) of body weight (Majumder, et al., 1959;

and Subramanyam et al., 1994). Also, the US Food and Drug Administration recognized the inert dusts as safe for use on grains (Banks and Fields, 1995;).

Kaolin is a naturally occurring, chemically inert clay mineral. It has been used to reduce negative impacts of environmental stresses on crop plants, to suppress diseases, and to protect crops from insect pests (Glenn and Puterka, 2005; and Kahn and Damicone, 2007). Kaolin and sand have been used traditionally as grain protectants by small-scale farmers in the developing world (Krishnakumari, 1964; Verma et al., 1976). Control of a variety of common storage insect pests with kaolin is demonstrated as effective as effective in conditions of low humidity when applied as dusts or in some cases when applied as water-based slurry (Golob, 1997). Adult populations of four stored grain insects infesting paddy treated with acid-activated kaolin at 0.75% w/w. controlled stored product insect pests up to 200 days post-treatment (Permual and le Patourel, 1990). The toxicity of acid-activated kaolin to adult populations of a number of insect species was assessed and determined effective at 72 h. *C. pusillus* and *O. surinamensis* were highly susceptible to the desiccant effect, while *T. castaneum* and the *Sitophilus* spp. were relatively tolerant. The susceptibility of all species to inert dusts decreased as paddy moisture content was increased from 7.2 to 19.2%. and F₁ progeny production was also reduced (Permual and le Patourel, 1990).

Mung bean seeds treated with inert dust resulted in 100% mortality of *C. chinensis* adults, also no adverse effect on germination was recorded for up to ten months of storage (Babbu et al., 1989). Adult *Trogoderma granarium* (Everts) exposed to rice treated with kaolin resulted in 100% mortality after 4 d at concentrations of 10000 ppm and after 2 months of exposure no offspring were observed as compared with 2800 insects produced by untreated beetles (Mostafa and Moagel, 1989). *C. maculatus* exposed to kaolin lead to 95% mortality of females and 100% of males with 0% mortality in the control after 6 h exposure (Keita et al., 2000). Kaolin has also been effective against both *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jacquelin du Val, and appears to have a potential for use in management programs to control beetles within storage facilities (Arthur and Puterka, 2002).

Kaolin may also be effective in controlling insects when used in combination with other components. Admixtures of isolates of entomopathogenic fungi *Beauveria bassiana* (BbGc and BbPs) with either kaolin, talc or tapioca flour (20% w/w a.i.) applied at 0.05 g/kg on long grain rice resulted in excess of 80% mortality of *Sitophilus oryzae* (L.) adults by the 7th day of exposure. A significantly higher grain weight loss was recorded with isolates mixed with tapioca flour, compared with that of kaolin or talc after 4 months of storage (Samodra and Ibrahim, 2006).

The aim of this study is to evaluate the protection performance of kaolin powder applied to broad beans against the insect pests *C. maculatus* and *C. chinensis*, and to determine if seed viability is impacted over a number of storage periods.

2. Materials and methods

2.1. Insect rearing

Callosobruchus maculatus and pulse *C. chinensis* stock cultures were maintained in 1-L glass jars containing *Vicia faba* and incubated in an environmental chamber at 30 ± 2 °C and 60 ± 5% relative humidity. Both insect species were reared in the research laboratory, Department of Plant Protection, Faculty of Agriculture, Al-Azhar University Cairo, Egypt for several generations. To distinguish between the insects, morphological characteristics are used. *C. maculatus* is a more elongate species with the posterior part of the abdomen not covered by the elytra which is more definitely spotted than *C. chinensis* (Hill, 1983). Differentiations of sexes were determined by examining the elytral pattern (Southgate et al., 1957; Halstead, 1963). Females are dark colored and possess four elytral spots. In contrast, males are pale brown and less distinguished by examination of the antennae, which are pectinate only in the male (Southgate, 1958;).

2.2. Broad bean seeds

The seeds of broad bean were collected from local stores, sieved and cleaned from dusts and inert materials. Seeds were frozen at -20°C for 48 h to kill previous infestations then kept under room conditions for 3 wks in order to reduce their moisture content to the normal rate (Huignard, 1985).

2.3. Treatment

Kaolin powder is a white nonabrasive fine-grained aluminum silicate mineral classified under, kaolinite group (OH)₈ AL₄ Si₄ O₁₀).

Dust was added to the beans at concentrations of 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025% w/w (powder/seeds) and were prepared by mixing kaolin powder manually with broad bean for each concentration and untreated control. Twenty broad bean seeds were taken out of the 200 g of treated seed, were weighed and then exposed to five pairs of newly emerged adults (0-2 day old) of both *C. maculatus* and *C. chinensis*. Insects were placed in small transparent glass jars and were kept in 30 ± 2 °C and 60 ± 5% r.h. covered with muslin for aeration. The trial was replicated four times for each tested concentration and storage period. The bioassays were carried out to verify the residual effect of kaolin dusts after 1, 3 and 6 months of storage on both insect species. After exposing the tested insects to treated seeds from three different storage periods (1, 3 and 6 months), determination of adult mortality, fecundity, hatchability, and F1 adult emergence were calculated. Treated and untreated broad bean weight loss was also calculated.

2.4. Biological studies and measured criteria

Parental adult mortality of the both tested insects in each tested concentration was calculated according to Abbott (1925). Fecundity was determined by counting the number of deposited eggs within each jar 7 d after removal of the adults. This time allowed the eggs to hatch and for larvae to penetrate the seeds. Hatched eggs were counted and test jars were monitored daily to determine F1 adult emergence. After adult emergence, seeds were weighed after excluding the frass and dust, and the percent weight loss was calculated using weight loss% equation (Khare and Johari, 1984) as following:

$$\text{Weight loss\%} = \frac{(\text{Initial dry weight} - \text{Final dry weight})}{\text{Initial dry weight}} \times 100$$

Reduction in fecundity, egg viability, F₁ adult emergence, and broad bean seed weight loss and seed viability of both tested insects treated with the kaolin powder were calculated according to Abbott (1925).

2.5. Kaolin application methods

An experiment was also performed to determine if there was a difference in effect of kaolin based on application of either a dust or a suspension. Dust application was performed as the method mentioned previously in the concentrations of 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025% w/w (powder/seeds). A kaolin suspension was applied by mixing the kaolin powder in distilled water to make a kaolin suspension of five concentrations; 2, 4, 6, 8 and 10% w/v (powder/water). A treatment of distilled water was applied as control. Broad beans were treated at each concentration by sub-merging 100 g of seeds for 30 sec and then aerating the beans over night for assuring complete dryness. Twenty broad bean seeds were taken from each 100 g treated seed at each concentration, were weighed and then exposed to five pairs of newly emerged adults (0-2 day old) of *C. maculatus* and *C. chinensis*. Each test was placed in small transparent glass jars and the trial was replicated four times for each concentration. Jars were kept at 30 ± 2 °C and 60 ± 5% r.h. covered with muslin for aeration.

2.6. Seeds germination

The viability of seeds treated with kaolin was also examined. Broad bean seeds previously treated with all tested concentrations of kaolin powder were stored at room conditions and tested after initial treatment and six month intervals of storage by placing 25 seeds on a cotton pad soaked with water in Petri dishes. The experiment was replicated four times for the treated and untreated beans and germination was observed and recorded.

2.7. Statistical analysis

Percent parental adult mortality, fecundity, egg viability, adult emergence of both insect species and percent of weight loss and viability of broad bean seeds treated with the kaolin were subjected to statistical analysis by Analysis of variance (ANOVA) test and linear regression analyses using a

computer software SAS (SAS Institute, 2000). Means were determined and compared by Duncan multiple range test at 0.05% probability level (Duncan, 1955).

3. Results

3.1. Influence of kaolin after different storage periods

3.1.1. Parental adult mortality

Beans treated with concentrations from 0.8 to 1.0% w/w of kaolin powder and exposed to insects after the first month of storage showed 100% mortality after six hours of exposure period to treated seeds. Concentrations of 0.6 to 1.0% w/w resulted in 100% adult mortality with seven days of exposure period. Insects exposed to kaolin treated beans after three months of storage also had 100% mortality at 0.8 and 1.0% w/w within 2 d (Table 1). Regression analysis indicate a positive correlation between the mortality percentage of exposed insects and the concentration of kaolin powder ($R = -0.74$) and ($R = 0.69$), respectively (Table 1). Results also revealed that after 6 mo of storage, seeds treated at 1.0% w/w concentration still showed a high level of adult mortality (66 and 73% compared to the untreated control 3.7 and 2.5%, for *C. maculatus* and *C. chinensis* respectively).

Table 1 Effect of kaolin powder on the fecundity, hatchability, life cycle, adult emergence and seeds weight loss of *Callosobruchus maculatus* and *C. chinensis* at different storage periods.

Storage Period	Conc. (%) w/w	Adult parents Mortality (%)		Number of eggs laid		Hatchability (%)		Number of F1 Emerged adults		Weight loss (%)	
		Macu-latus	Chinen-sis	Macu-latus	Chine-n-sis	Macu-latus	Chine-n-sis	Macu-latus	Chine-n-sis	Macu-latus	Chinen-sis
Month 1	1	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.8	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.6	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.4	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.2	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.1	68	78	13	10	32	23	1.7	0.7	1.2	0.7
	0.05	46	57	38	25	44	45	6.78	5	3.6	4.7
	0.025	19	37	68	40	66	55	20	8.	9.9	8.2
	Ave.	79	84	15	9.37	17.78	15	3.6	1.7	1.9	1.7
	Control	5	5	373	283	92.4	87.14	212.5	23.02	30	23.
	R-value	0.7	0.7	-0.5	-0.5	-0.7	-0.7	-0.4	-0.5	-0.5	-0.5
	Slope	14.9	12.9	-153.7	-112.4	-69.3	-62.5	-76.9	-11.3	-13.9	-11.3
Month 2	1	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
	0.8	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	0.6	82	56	19	16	33	38.	8	2	4.4	2.6
	0.4	68	44	37	45	44	48	19	10	6.3	9.1
	0.2	56	33	60	58	56	57	26	14	9	13.7
	0.1	38	26	86	80	65	66	36	22.	14	16.9
	0.05	19	18	112.	115	72	71	55	48.	20	21.5
	0.025	3	8	197	190	81	78	88	78	23	25.1
	Ave.	58	48	64	63	44	45	29	22	10	11.1
	Control	2.5	2.5	325.	298	92	89	217.5	167	33	27.5
	R-value	0.9	0.97	-0.79	-0.8	-0.9	-0.9	-0.97	-0.7	-0.8	-0.9
	Slope	19.3	18.8	-250.2	-205.8	-87	-84.1	-87.5	-97.2	-25.7	-27.2
Month 3	1	66	73	50	45	50	40	9	6.8	3	4.1
	0.8	59	55	63	55	58	48	15	13	8	7.6
	0.6	49	42	83	80	68	56	24	18	12	11.5
	0.4	38.9	32	102	98	69	60	34	28	16	16.5
	0.2	32	24	130	132	67	61	55	45	21	19.4
	0.1	27	17	160	170	79	70	78	55	23	22.4
	0.05	21	10	220	243	84	81	113	90	24	24.7
	0.025	6.5	6	258	265	86	88	167	130	26	26.7
	Ave.	33	33	133	136	70	63	62	48	16	16.6
	Control	3.7	2.5	320	298	92	90	215	175	27	28.5
	R-value	0.9	0.9	-0.9	-0.9	-0.9	-0.9	-0.8	-0.8	-0.9	-0.9
	Slope	10.9	12.7	-218	-229	-35	-43.7	-158	-125.1	-23.7	-23

When data from all storage periods are averaged, the percent mortality of adult *C. maculatus* and *C. chinensis* increased significantly ($P=0.062$) as concentration of kaolin on broad beans (56.92 and 54.82% compared to the untreated 3.75 and 3.33%, Table 2).

Table 2 Effect of kaolin powder on the fecundity, hatchability, life cycle, adult emergence and seeds weight loss of *Callosobruchus maculatus* and *C. chinensis*.

Concentration (%) w/w	Adult parents mortality (%)		Number of eggs laid		Hatchability (%)		Number of F1 Emerged adults		Weight loss (%)	
	<i>Macu- latus</i>	<i>Chinen- sis</i>	<i>Macu- latus</i>	<i>Chinen- sis</i>	<i>Macu- latus</i>	<i>Chinen- sis</i>	<i>Macu- latus</i>	<i>Chinen- sis</i>	<i>Macu- latus</i>	<i>Chinen- sis</i>
1	88.74	91.02	16.66	15.00	16.76	13.40	2.91	2.25	1.03	1.37
0.8	85.28	85.04	20.83	18.33	19.48	15.83	5.00	4.16	2.58	2.54
0.6	77.13	66.23	33.91	32.08	33.56	31.57	10.50	6.33	5.35	4.70
0.4	68.96	58.54	46.66	47.50	37.63	36.15	17.41	12.33	7.27	8.55
0.2	62.95	52.56	63.33	63.33	41.19	39.31	27.08	19.58	9.86	11.05
0.1	44.71	39.97	86.25	86.66	58.94	52.86	38.50	26.08	12.76	13.37
0.05	28.68	28.25	123.33	127.5	66.68	65.45	58.08	47.75	16.03	16.96
0.025	09.59	16.98	174.16	165.0	77.72	73.73	91.66	71.75	19.72	19.98
Control	03.75	3.33	339.16	292.5	92.17	88.66	215.00	175.0	29.91	26.33
F-value	55.40		35.59		51.13		32.79		2.88	
P=0.05	0.0001		0.001		0.0001		0.0001		0.005	

3.1.2. Fecundity

Concentrations from 0.2 to 1.0% w/w of kaolin powder was clearly effective in reducing egg production by both insect species the first month of storage as exposed adults died within a few hours. After 3 months of storage, only concentrations of 0.8 to 1.0% v/w gave a 100% protection for both tested insects (Table 1). Regression analysis indicates a negative correlation between the number of eggs laid and the concentration of kaolin ($R= -0.468$) and ($R= -0.451$), for *C. maculatus* and *C. chinensis* respectively (Table 1). Results also indicate that after six months of storage, seeds treated with 1.0% w/w concentration have a persistent effect as a seed protectant against bruchid fecundity where eggs laid averaged 50.00 and 45.00 eggs/female compared to 320.00 and 298.00 eggs/female in controls for *C. maculatus* and *C. chinensis*, respectively.

Table 3 Residual effect of Kaolin powder on *Callosobruchus maculatus* and *C. chinensis* after three different storage periods of treated and untreated broad bean seeds.

Treatment	Storage period (months)	Adult parents mortality (%)		Number of Eggs laid		Hatchability (%)		Number of F1 Emerged adults		Weight loss (%)	
		<i>Macu- latus</i>	<i>Chinen- sis</i>	<i>Macu- latus</i>	<i>Chinen- sis</i>	<i>Macu- latus</i>	<i>Chine- n-sis</i>	<i>Macu- latus</i>	<i>Chinen- sis</i>	<i>Macu- latus</i>	<i>Chinen- sis</i>
Treated	1	79.27	83.88	14.68	9.37	17.77	15.43	3.56	1.69	1.853	1.694
	3	58.33	48.07	64.12	62.96	43.85	44.83	28.90	21.68	9.707	11.152
	6	33.17	32.53	133.12	135.93	70.36	62.85	61.71	48.03	16.426	16.613
	Average	56.92	54.82	70.64	69.42	43.99	41.04	31.39	23.80	9.328	9.819
Untreated	1	5.0	5.0	372.50	282.50	92.39	87.14	212.50	23.02	30.089	23.024
	3	2.5	2.5	325.00	297.50	92.15	89.07	217.5	167.5	32.651	27.485
	6	3.75	2.5	320.00	297.50	91.97	89.77	215.00	175.00	27.004	28.503
	Average	3.75	3.33	339.16	292.50	92.17	88.66	215.00	121.84	29.914	26.337
F-value		2.83		0.30		0.90		0.30		0.00	
P-value		0.062		0.0001		0.0001		0.05		0.0001	

The impact of kaolin powder on the fecundity of both insect species regardless of concentration significantly reduces the number of eggs laid ($P=0.0001$) on the treated broad beans (Table 3). The reduction of mean eggs laid over the three storage periods averaged 78.0 – 76.0%, for *C. maculatus* and *C. chinensis* respectively (Table 4).

3.1.3. Egg viability

No eggs were laid by insects exposed to beans treated with concentrations from 0.2 to 1.0% w/w of kaolin powder. Regression analysis indicates a negative correlation between the percent hatchability and

the concentration of kaolin powder (Table 1). Results also reveal that after six months of storage, seeds treated at 1.0% w/w have a persistent effect as a seed protectant against bruchid fecundity averaging 50 and 40 egg/female compared to the untreated control 92 and 90 egg/female, respectively for *C. maculatus* and *C. chinensis*. The viability of eggs produced by both species decreased significantly ($P=0.0001$) and ($P=0.0005$) when adults were placed on treated broad beans (44.0 and 41.0%) compared to the untreated (92.1 and 88.7%) in all periods (Table 2). Consequently, the reduction of egg viability reached to 80% and 82% in the first month of storage and was reduced about 50% after 3 and 6 months (Table 3). In general, hatchability was over 50% for both insect species (Table 4).

Table 4 Influence of kaolin powder on *Callosobruchus maculatus* and *C. chinensis* biological aspects after different storage periods.

Storage period (months)	Reduction % of Number of eggs Laid		Reduction % of hatchability		Reduction % of F1 emerged Adults		Reduction % of weight loss	
	<i>Maculatus</i>	<i>Chinensis</i>	<i>Maculatus</i>	<i>Chinensis</i>	<i>Maculatus</i>	<i>Chinensis</i>	<i>Maculatus</i>	<i>Chinensis</i>
1	96.0	96.0	80.0	82.0	98.0	92.0	93.84	92.64
3	80.0	78.0	52.0	49.0	86.0	87.0	70.27	59.42
6	58.0	54.0	23.0	29.0	71.0	72.0	39.17	41.71
Average	78.0	76.0	52.0	53.0	85.0	84.0	67.76	64.59

3.1.4. *F₁* adult emergence

Overall, the number of F1 adults produced by both insect species increases as treatment concentration decreases and as storage time is increase. Survival of F₁ adults of both insect species decreased significantly ($P=0.0001$), in the treated broad bean beans averaging 31.39 and 23.80 adults compared to the untreated 215.00 and 121.84 for all storage periods (Table 3). Reduction of F1 adults reached to 98% and 92% for *C. maculatus* and *C. chinensis* for the first month of storage while F1 adults were reduced from 70 to 87% for 3 and 6 months of storage (Table 4). Generally, the potential effect of kaolin reduced of F1 emergence by approximately 85.00 and 84.0% for both tested insects, respectively (Table 4).

3.1.5. Seeds weight loss

There was no weight loss of beans observed at higher application rates of kaolin and at shorter storage durations, (Table1) and regression analysis indicates a negative correlation between the weight loss and the concentration of kaolin ($R= -0.52$) and ($R= -0.55$), respectively for the insect species (Table 1). *Callosobruchus maculatus* and *C. chinensis* caused a significant weight loss to the broad beans regardless to the concentration of kaolin ($P=0.0001$). Treated broad bean were reduced by approximately 9.5% compared to the untreated where weight loss averaged 28% for the untreated beans over all storage periods (Table 3). The first month of storage showed the least bean weight loss (1.7%) for treated beans while untreated beans lost an average of up to 93%. The weight loss of treated beans for the other two storage periods (3 and 6 months) was relatively lower, ranging from 39 to 70% (Table 4). Generally, kaolin application reduced the weight loss with the average mean of 67.76 and 64.59% for the insect species, respectively (Table 4).

3.2. Kaolin application methods

Kaolin application methods were compared by exposing the two insect species to treated broad bean seeds with kaolin powder or kaolin suspension. Results in Table 5 illustrated that kaolin powder was more effective than kaolin suspension in all criteria tested. The number of eggs laid, F1 adults and seed weight loss were all greater for the dust application than the suspension application (Table 5).

Table 5 Comparison of kaolin application methods on the biological aspects of *Callosobruchus maculatus* and *C. chinensis*.

Tested insects	Treatment	Number of eggs laid		F1 emerged adults		Seeds weight loss (%)	
		Suspension	Powder	Suspension	Powder	Suspension	Powder
<i>maculatus</i>	Treated	138.25	70.64	63.85	31.39	15.34	9.32
	Untreated	340.00	345.00	230.00	235.0	24.75	26.25
<i>chinensis</i>	Treated	116.00	69.42	47.75	23.80	12.341	9.82
	Untreated	252.50	292.5	90.41	121.8416	15.75	26.33
P=0.05		0.022		0.008		0.014	

3.3. Seed viability

The effect of kaolin powder treatment on broad bean seeds germination is presented in Table 6. Results showed slight differences between untreated and treated beans in the germination percentage at all tested concentrations. Germination decreased in the untreated seeds from 92 for the initial time to 81.33% after six months of storage period. The general mean of the germination reduction% of the two tested storage periods regardless to the concentration averaged 2.04 and 3.02%, respectively. In conclusion, while there is a trend, kaolin application has no adverse effect on broad bean viability.

Table 6 Effect of kaolin powder on germination of treated broad bean seeds at two different storage periods.

Conc.(%) w/w	Initial time		After 6 months	
	Germination(%)	Reduction (%)	Germination (%)	Reduction (%)
1	88.66	3.63	77.00	5.32
0.8	89.33	2.90	77.00	5.32
0.6	89.00	3.26	78.33	3.69
0.4	89.33	2.90	78.66	3.28
0.2	90.33	1.81	79.00	2.87
0.1	90.66	1.46	79.66	2.05
0.05	91.66	0.37	80.33	1.23
0.025	92.00	0.00	81.00	0.41
Average	90.12	2.04	78.88	3.02
Control	92.00		81.33	
R-value	-0.90		-0.94	
Slope	-3.23		-4.04	

4. Discussion

The effectiveness of kaolin powder as a control measure against *C. maculatus* and *C. chinensis* is clearly apparent. At high concentrations, adult mortality, fecundity, egg viability and F1 adults were all affected by kaolin. Kaolin powder at concentrations of 8000 and 10,000 ppm provides protection for broad bean seeds for up to 6 months (Table 3). These results agreed with Strong and Sbur (1963) who reported wheat kernels were completely riddled by the end of 6 months after exposing to *Sitophilus oryzae*. While, treated wheat with inert dusts would prevent infestations for 6 months. Persistence of inert dusts has been demonstrated effective even after 3 and 6 months of storage by maintaining parental mortality and reducing progeny emergence for 4 stored grain insects (Stathers et al., 2002). Kaolin application controlled four stored grain insects up to 200 days post-treatment (Permual and le Patourel, 1990). The effectiveness of kaolin on newly emerged adults of *T. granarium* mixed with rice grains demonstrated 100% mortality after 4 d of treatment at 10000 ppm. After two months of exposure parents did not produce any offspring as compared with 2800 insects produced by the untreated beetles (Mostafa and Moagel, 1989). A significantly higher grain weight loss was recorded with isolates of *Beauveria bassiana* mixed with tapioca flour, compared with that of kaolin or talc after 4 months of storage (Samodra and Ibrahim, 2006). Prolonging storage period reduces the effectiveness of kaolin powder against the two tested insects, likely due to increasing the moisture content of the kaolin particles. This leads to changes in the physical properties that reduces the kaolin particles efficiency to disrupt the insect cuticle.

Results in Table 4 indicated that reduction of the tested biological aspects reached highest values in the first month of the storage period, then declined gradually as the storage period prolonged. Mahdi and Khalequzzaman (2006) found that the efficacy of inert dusts against insects depends greatly on the physical properties of the dust. These materials prevent insect infestations forming natural physical barrier deters insect infestation and impedes their movement, feeding and egg laying (Shadia abd El-Aziz, 2003).

Kaolin powder application showed high performance in protection of seed against bruchids than the kaolin suspension application. This may be due to the changes of physical properties of kaolin particles as a result of mixing with water. Consequently, kaolin particles get soft, and this impacts its ability to disrupt the insect cuticle. Our results show that the number of eggs laid, F1 emerged adults and seed weight loss was clearly higher in the powder application than in suspension application. Kaolin powder offers unsuitable surface for laying eggs for the female than the surface treated with suspension and

reduces the number of eggs laid by approximately half. Kaolin applications are most effective in conditions of low humidity, also when applied as dusts but some retain activity even when applied as water-based slurry (Golob, 1997). The efficacy of inert dusts is very sensitive to grain moisture content (Permul and le Patourel, 1990). Application of kaolin powder led to 95% mortality of *C. maculatus* females and 100% of males with 0% of mortality in the control after 6 h exposure (Keita et al., 2000). Treated mung bean with kaolin powder resulted in a 100% mortality of *C. chinensis* adults (Babbar et al., 1989).

Kaolin application had very slight adverse effect on broad bean seeds germination in the highest concentration, and no adverse effect on the low concentration of kaolin. Babbar et al. (1989) reported that mung bean mixed with kaolin powder had no any adverse effect on treated mung bean seeds germination for up to 10 months of storage period. Treated broad bean seeds with kaolin powder showed slight adverse effect seed viability for up to 6 months of storage period. Therefore, the use of kaolin inert dusts should be encouraged as stored grain protectant due to its effectiveness and being non-toxic material.

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