

*Hamid SAKENIN CHELAV,*  
*Adel KHASHAVEH, Farshid SHAKHSI ZARE*<sup>1</sup>

**ADULT MORTALITY AND PROGENY PRODUCTION  
ASSESSMENT OF *Callosobruchus maculatus*  
(COLEOPTERA: BRUCHIDAE) EXPOSED TO SAYAN®**

**SUMMARY**

The insecticidal efficacy of Sayan®, an Iranian formulation of diatomaceous earth, was evaluated against adults of Cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae) on cowpea. Sayan® was applied at the dose rates of 0.0, 0.1, 0.2, 0.5, 1.0 g/kg. One kg of cowpea was treated with appropriate dose rate and after one day, four samples of 50 g of each were taken as replication. Twenty five adults were introduced into each replication. The number of dead adults was counted after 24 and 72 h exposure. After mortality count, all surviving insects were removed and the samples retained under the same conditions for a further 35 days to assess progeny production. For adult's mortality, significant differences were found between dose rates, exposure times and associated interaction. The percentage of cowpea weevil adult's mortality was very low at the dose rates of 0.0, 0.1 and 0.2 g/kg (less than 5%) after both exposure times. The mortality rate increased with increase of dose rate and exposure time and reached 68% at the dose rate of 1.0 g/kg 72 h after exposure. Also, for progeny production, significant differences were found between dose rates, exposure times and associated interaction. In both exposure times, the highest number of F<sub>1</sub> progeny was recorded at 0.0 g/kg and increasing in dose rate significantly decreased the progeny production. The obtained results clearly revealed that Sayan® is effective against *C. maculatus* but higher dose rates and exposure time are needed to achieve satisfactory control of this pest in cowpea.

**Key words:** *Callosobruchus maculatus*, Cowpea, Sayan®, adult mortality, progeny production

**INTRODUCTION**

Legumes (family Fabaceae or Leguminosae) are made up of many species, which are cultivated all over the world for humans and animals consumption. They also provide nitrogen to the soils (Ofuya and Akhidue, 2005; Umeozor, 2005). Cowpea, *Vigna unguiculata* (L.) Walp, is mainly grown in tropical and

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<sup>1</sup> Hamid Sakenin Chelav, Department of Plant Protection, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran, Adel Khashaveh (corresponding author: adel.khashaveh@gmail.com), Young Researchers and Elites Club, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran, Farshid Shakhsi Zare, Department of Entomology, Agricultural Campus, Islamic Azad University, Arak, Iran

subtropical regions in the world for vegetable and seed purpose. Being the rich source of potent nutritional elements, the tender, soft and green pods of cowpea are used as vegetable and other aerial plant parts are utilized as green fodder for domesticated cattle. It provides more than half the plant protein in human diets and it is the key source of protein for the poorest sector of many developing countries (Remya, 2007).

The production of cowpea in many regions is restricted by a number of biotic and abiotic factors, both in the field and the seed in storage. Insect pests are among the constraining biotic factors (Swella and Mushobozy, 2007). One of the most harmful insect species is cowpea weevil. Cowpea weevil, *Callosobruchus maculatus* (F.) Walp (Coleoptera: Bruchidae) is a cosmopolitan pest species of cowpea in the tropics and subtropics of the world and an important field-to-store pest of legume crops in Africa and Asia. Infestation usually occurs in the store, but the adult beetle can fly up to half a mile so that field crops within this distance are likely to be infested. In this way, this bruchid can attack legumes when they are still in the field (Iloba and Umoetok, 2007). Females cement their eggs to the surface of host seeds. And after a few days, the eggs hatch. The larval stage of the weevil makes tunnel and develops within the cowpea seeds. It causes substantial quantitative and qualitative losses, manifested by seed perforation, and reductions in weight, market value and germinability of seeds. Under traditional storage conditions, 100% infestation of cowpea, occurring within 3–5 months of storage, is common (Fox and Tatar, 1994; Lale and Mustapha, 2000; Parsaeyan et al., 2012).

Fumigants and residual chemical insecticides such as phosphine, methyl bromide, deltamethrin and malathion are used for controlling *C. maculatus*. The use of fumigants and conventional insecticides caused the development of pesticide resistance, hazardous effects to non-target species and beneficial organisms, and environmental and food contamination. Therefore, it is necessary to find out appropriate alternative materials with safety and low toxicity characteristics to human and environment (Khashaveh et al., 2011; Parsaeyan et al., 2012). One alternative is diatomaceous earth (DE), the fossilized remains of diatoms that form an inert dust (Kavallieratos et al., 2010). Different formulations of DEs that are commercially available, have been long known as a potentially useful grain protectant because it is safe to use, does not affect grain end-use quality, provides long-term protection and is comparable in cost to other methods of grain protection. It is composed almost entirely of amorphous silicon dioxide (SiO<sub>2</sub>) (Ziaee and Khashaveh, 2007; Ziaee et al., 2007; Beris et al., 2011).

DEs are extremely porous and when insects come into contact with small particles of this dust, the waxy layer of the cuticle is absorbed resulting in water loss, desiccation and death (Subramanyam and Roesli, 2000; Stathers et al., 2004). They have extremely low toxicity to mammals (e.g. DE rat oral LD<sub>50</sub>>5000 mg/kg) (Subramanyam et al., 1994) and are registered for use as grain protectants in many countries such as USA, Australia, Brazil, Canada,

Croatia, China, Germany, Indonesia, and Japan. These alternative materials are regarded as “Safe” by USA Environmental Protection Agency (Stathers et al., 2004). In 2011, DEs are registered in Iran by Plant Protection Organization for direct application to stored grain and storage facilities.

Efficiency of DEs against cowpea weevil was first reported by Rohitha Prasantha (2002 a, b) but then, many researchers have worked in this field and their results showed that DEs are considerably able to control this pest and other *Callosobruchus* species (Stathers et al., 2004; Remya, 2007; Islam et al., 2010; Wakil et al., 2010; Wakil et al., 2011; Parsaeyan et al., 2012).

The objective of present study was to evaluate the efficacy of Sayan® (a newly produced and registered DE formulation in Iran) against adults and reproductive capacity of *C. maculatus* on cowpea.

## MATERIAL AND METHODS

### *Insect rearing*

Insects were obtained from laboratory cultures. The cultures had been maintained at the Laboratory of Entomology, Department of Plant Protection, Islamic Azad University, Qaemshahr Branch, for more than one year, with no history of exposure to insecticides. *C. maculatus* was reared on cleaned and disinfested cowpea seeds at  $27\pm 1^\circ\text{C}$  and  $60\pm 0.5\%$  relative humidity (RH) in continuous darkness. All adults used in the experiments were 0–24 h old and of mixed sex. For getting 0–24 h old adults, seeds with pupa window were separated and after one day, adults that emerged were collected with a hand-made aspirator.

### *DE formulation*

Sayan®, a product of Kimia Sabz Avar Co, Tehran, Iran, is a light gray to off-white powder that contains 80% silicon dioxide ( $\text{SiO}_2$ ). The median particle size is between 10 and 20  $\mu\text{m}$ . This DE formulation is commercially available.

### *Adult mortality assessment*

DE formulation was applied at five rates: 0.0, 0.1, 0.2, 0.5, 1.0 g/kg and mortality was recorded after 24 and 72 hours exposure. Therefore, ten lots of 1 kg of cowpea seed (one lot for each dose rate-exposure time) were prepared and placed in separate cylindrical jars (3-L capacity with screwed lids) and treated with appropriate dose rates. Subsequently, the jars were shaken manually for approximately 2 min to obtain an even distribution of DE on the entire seed mass. After a day, four samples of 50 g, were taken from each lot as replication and then placed in glass vials (8 cm height and 5 cm diameter). Immediately, 25 adults were introduced into each glass vial that was covered with muslin cloth to provide sufficient aeration. The vials were then placed in incubators, set at  $27\pm 1^\circ\text{C}$  and  $60 \pm 0.5\%$  RH, in darkness. The number of dead adults was counted after 24 and 72 h exposure.

### *Progeny production assessment*

Mortality counts were made by pouring the contents of each vial into the tray. After the mortality count, all adults (dead and alive) were removed from the tray and the entire contents were returned into each vial. The vials were left in the incubators at  $27\pm 1^\circ\text{C}$  and  $60\pm 0.5\%$  RH, in darkness for an additional period based on the life cycle of cowpea weevil. Progenies emergence started after day 28. Emerged adults in each vial were counted daily up to 35 days after introduction to avoid overlapping of generations.

### *Statistical analysis*

Prior to submission of data to the statistical software for analysis of variance, the normality test was carried out by MINITAB 14 to examine whether or not the data follow a normal distribution (Ryan et al., 2005) and because adult mortality and progeny production data were not normally distributed, mortality percentage of adults and number of progeny production were transformed using arcsine square-root and  $\log x$ , respectively, to equalize variances. The mortality data were analyzed by the General Linear Model (GLM) of the Statistical Analysis System (SAS Institute Inc., 2004); with insect mortality as the response variable and dose rate and exposure time used as main effects. The same procedure was also followed for progeny production counts. Means were separated by using the Duncan's multiple range test at  $P = 0.05$  (Frey, 2010). The dose rate required to kill 50% ( $LD_{50}$ ) and 95% ( $LD_{95}$ ) of the insects were estimated using probit analysis (SPSS, 1999).

## RESULTS AND DISCUSSION

### *Adult's mortality*

For adults mortality, significant differences were found between main effects (exposure time and dose rate) and associated interaction (exposure time  $\times$  dose rate). The details are given in table 1. The percentage of cowpea weevil adult's mortality was very low at the dose rates of 0.0, 0.1 and 0.2 g/kg (less than 5%) after both exposure times. The mortality rate increased with increase of dose rate and exposure time. The percentage of mortality after 24h of exposure did not exceed 30% even at the rate of 1 g/kg but reached 68% after 72h exposure (figure 1).

Table 1- ANOVA parameters for main effects and associated interaction for adult mortality

Source	df	F value	P
Exposure time	1	4.49	0.0424
Dose rate	4	10.20	<0.0001
Exposure time $\times$ Dose rate	4	5.52	0.0019
Error	30		
Corrected total	39		

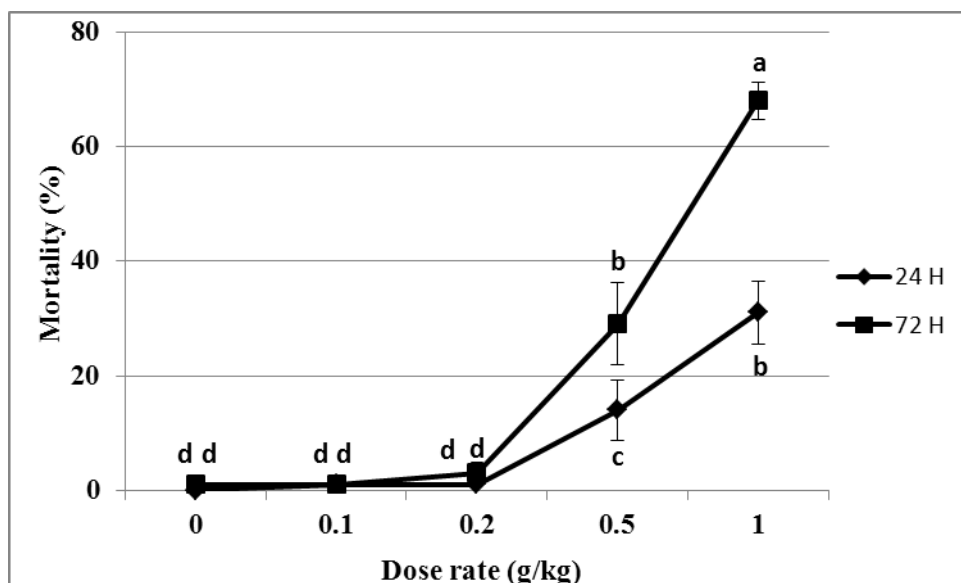


Figure 1- Mean mortality ( $\pm$ SE) of *C. maculatus* adults exposed for 24 h and 72 h in cowpea treated with different dose rate of Sayan<sup>®</sup>. Means followed by the same letter are not significantly different

The linear relationship between the exposure times and adults' mortality was attained using probit analysis. The test for the goodness of fit indicates no significant heterogeneity in the linear relationships, for both exposure times tested (small  $\chi$  values,  $p > 0.05$ ). Based on the estimations of LD<sub>50</sub> and LD<sub>95</sub>, and associated 95% confidence limits and no observed overlap, there was significant difference between the two exposure times. The details of probit analysis are shown in table 2.

Table 2- Probit analysis data for *C. maculatus* adults in cowpea treated with Sayan<sup>®</sup> after 24 and 72 hours of exposure

Exposure time	LD <sub>50</sub> (g/kg)	95% CL*	LD <sub>95</sub> (g/kg)	95% CL	Intercept (a)	Slop (b)	P	$\chi^2$
24 h	1.69	(1.25-2.85)	9.93	(4.98-16.48)	- 0.487	2.14	0.432	1.68
72 h	0.72	(0.64-0.84)	2.43	(1.83-3.56)	0.432	3.15	0.427	1.70

\* Confidence limit

### Progeny production

For progeny production, significant differences were found between main effects (exposure time and dose rate) and associated interaction (exposure time  $\times$  dose rate). The details are given in table 3. In both exposure times, the highest number of progeny ( $F_1$ ) was recorded at 0.0 g/kg ( $95.25 \pm 6.60$  and  $199.50 \pm 8.70$  after 24h and 72h exposure to treated cowpea seeds, respectively) and increase in dose rate significantly decreased the progeny production (figure 2).

Table 3- ANOVA parameters for main effects and associated interaction for progeny count

Source	df	F value	P
Exposure time	1	101.39	<0.0001
Dose rate	4	21.39	<0.0001
Exposure time $\times$ Dose rate	4	3.22	0.0258
Error	30		
Corrected total	39		

## DISCUSSION

The first official report about the potential of Sayan<sup>®</sup> for control of storage pest was published by Shakhshi Zare (2013). He assessed the efficacy of this new formulation of DE on red flour beetle (*Tribolium castaneum*) and saw-toothed grain beetle (*Oryzaephilus surinamensis*). The obtained results by this researcher demonstrated that the performance of this formulation is acceptable and complete mortality (100%) was noted for adults of red flour beetle and saw-toothed grain beetle at the dose rate of 2 g/kg for 30 days after exposure and dose rate of 1.5 g/kg for 14 days after exposure, respectively. These results showed that Sayan<sup>®</sup> had better efficacy on *O. surinamensis* than *T. castaneum*. Our study is the first research work that evaluated the property of Sayan<sup>®</sup> for control of cowpea weevil and there is not any references regarding Sayan<sup>®</sup> and different stored pulses insects to be compared with the results obtained in the current work but it can be compared to results from other researchers who worked with other formulation of DEs and stored pulses pests.

Many researchers worked with different formulations of DEs and according to conditions of experiments (temperature and relative humidity), the population of insect, proficiency of formulation (the medium particle size and percentage of SiO<sub>2</sub> in formulation) and types and varieties of commodities, the obtained results were different. But all previous investigations demonstrated that DEs are effective against cowpea weevil. Wakil et al (2010) indicated that DiaFil 610 at 30°C and 50% RH and dose rate of 0.2 g/kg caused about 50% in adults of *C. maculatus*. At the same conditions, they recorded 100% mortality when exposure time and dose rate increased to 5 days and 0.8 g/kg, respectively. SilicoSec<sup>®</sup> at the dose rates of 0.5, 1, 1.5 and 2 g/kg was applied against cowpea weevil at three varieties of cowpea. The obtained results showed that the greatest

rate of SilicoSec<sup>®</sup> (2 g/kg) caused 100% adults mortality in all three varieties 4 days after exposure (Tofel et al., 2012). In our experiments, highest rate of adult's mortality of cowpea weevil was about 68% after 72 h exposure at the dose rate of 1 g/kg. So, the use of higher dose rates (1.5 or 2 g/kg) of Sayan<sup>®</sup> and more exposure times (5 days) are recommended to obtain maximal mortality. Rohitha Prasantha et al. (2002b) indicated that adult's mortality of cowpea weevil exposed to Fossil-Shield<sup>®</sup> at the dose rate of 1.02 g/kg of mung bean, are significantly dependent on temperature and relative humidity and highest mortality (nearly 100%) was achieved at 35°C and 43% RH. In conditions close to our works, these investigators reported about 60% mortality at mentioned dose rate. They proved that mortality rate decreases with increasing temperature and decreasing relative humidity. Therefore, further experiments with different conditions of temperature and relative humidity are needed to exactly determine the efficiency of Sayan<sup>®</sup>.

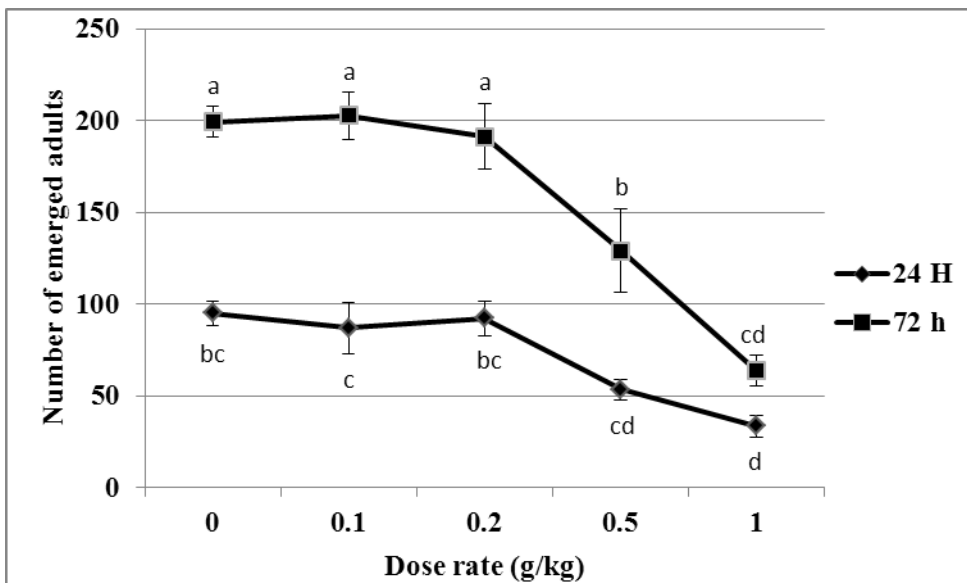


Figure 2- Progeny production (mean number of adults/Vial $\pm$ SE) of *C. maculatus* exposed for 24 h and 72 h in cowpea treated with different dose rate of Sayan<sup>®</sup>. Means followed by the same letter are not significantly different (Separated by Duncan's multiple range test at  $P = 0.05$ )

The toxicity study of SilicoSec<sup>®</sup> against *C. maculatus* was done by Mahdi and Khaleqzaman (2006). The calculated LD<sub>50</sub> value for 24h and 48h exposure was 0.737 and 0.385 g/kg, respectively. Also, insecticidal efficacy of SilicoSec<sup>®</sup> on *C. maculatus* based on LD<sub>50</sub> and LD<sub>95</sub> was presented at the work of Shams et al. (2011). These researchers reported that the dose rates of 0.352 and 0.674 g/kg of SilicoSec<sup>®</sup> were needed to obtain 50 and 95% adult mortalities (respectively) after 24 h but these amounts decreased with increased time of exposure and after 48h exposure, the LD<sub>50</sub> and LD<sub>95</sub> values were 0.300 and 0.504 g/kg, respectively.

These results confirm our works and explain that the lethal dose for 50% and 95% of insect population exposed to DEs, decrease with increase of exposure time.

Suppression of the subsequent generations is one of the basic characteristics of a successful grain protectant (Arthur, 1996) and considered as equal as parental mortality or even more important. Our experiments demonstrated that Sayan<sup>®</sup> reduced the number of emerged adults in F<sub>1</sub> generation in treated cowpea. At the dose rate of 0.0 g/kg and 72h exposure, average number of emerged adults per vial was 199.5±8.70. Increase of dose rate to 1 g/kg decreased the average number of emerged adults to 63.75±8.20 per vial. However, the high progeny emergence of *C. maculatus* adults suggests that higher application rates of this formulation should be tested against these species. Since parental mortality rate was low after 24h exposure time, it was expected to produce more progeny. But as seen in figure 2, the insects that exposed to Sayan<sup>®</sup> for 24h produce fewer progeny than those exposed for 72h. This result can be interpreted that the insects exposed to the Sayan<sup>®</sup> for 24h, had less time for mating and egg-laying. Rohitha Prasantha et al. (2002a) proved that for SilicoSec<sup>®</sup>, number of emerged adults (F<sub>1</sub> progeny) from the treated group and control were not significantly different at the dose rates of 0.1, 0.2 and 0.4 g/kg of mung beans. These results confirm our results that DEs at low dose rates could not control F<sub>1</sub> progeny production of cowpea weevil.

### CONCLUSIONS

As main conclusions, the obtained results from the adult's mortality assessment clearly revealed that Sayan<sup>®</sup> is effective against *C. maculatus* but higher dose rates and exposure time are needed to achieve satisfactory to complete control of this pest in cowpea. Higher dose rates could restrain the production of progeny in treated cowpea, too. Since Sayan<sup>®</sup> is a new product of DE, little information is available regarding its efficacy. Therefore, further tests are recommended to be done about the accurate performance of this formulation in different legumes. Also, further study is needed to evaluate the long term persistence of Sayan<sup>®</sup> for control of different stored legume pests.

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*Hamid SAKENIN CHELAV,  
Adel KHASHAVEH, Farshid SHAKHSI ZARE*

**PROCJENA MORTALITETA ODRASLIH JEDINKI I PRODUKCIJA  
POTOMSTVA *Callosobruchus maculatus* (COLEOPTERA: BRUCHIDAE)  
IZLOŽENIH SAYAN®**

**SAŽETAK**

Insekticidna efikasnost Sayan®, iranske formule dijatomejske zemlje ocjenjivana je u odnosu na odrasle jedinke žiška stočnog graška *Callosobruchus maculatus* (Coleoptera: Bruchidae) na stočnom grašku. Sayan® je primijenjivan u dozama od 0,0; 0,1; 0,2; 0,5; 1,0 g/kg. Jedan kg stočnog graška tertian je odgovarajućom dozom i nakon jednog dana, uzeta su četiri uzorka od 50 g kao replikacija. Dvadeset pet odraslih jedinki je uvedeno u svaku replikaciju. Brojanje mrtvih odraslih jedinki vršeno je nakon izlaganja tokom 24 i 72 časa. Nakon brojanja uginulih, svi preživjeli insekti su uklonjeni a uzorci su držani pod istim uslovima narednih 35 dana kako bi se izvršila procjena produkcije potomstva. Kod mortaliteta odraslih jedinki, utvrđene su značajne razlike zavisno od primijenjene doze, vremena izlaganja i sa pratećim interakcijama. Mortalitet žiška stočnog graška je bio veoma nizak prilikom doza od 0,0; 0,1 i 0,2 g/kg (manje od 5%) nakon oba vremena izlaganja. Stopa mortaliteta rasla je sa povećanjem doze i vremena izlaganja i dostigla je 68% pri dozi od 1,0 g/kg 72 časa nakon izlaganja. Takođe, kada je u pitanju produkcija potomstva, otkrivene su značajne razlike između doza, vremena izlaganja i pratećih interakcija. U oba vremena izlaganja, najveći broj F1 potomstva zabilježen je kod 0,0 g/kg i povećanje primijenjene doze značajno je smanjilo produkciju potomstva. Dobijeni rezultati su jasno pokazali da je Sayan® djeltvoran protiv *C. maculatus* ali su više doze i duže vrijeme izalganja potrebni da bi se postiglo zadovoljavajuće suzbijanje ove štetočine u stočnom grašku.

**Ključne riječi:** *Callosobruchus maculatus*, stočni grašak, Sayan®, mortalitet odraslih jedinki, produkcija potomstva