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Evaluation the efficiency of five inorganic salts on the cowpea beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

Mahmoud, M.A.[1]; Omar, Y.M. [2]; Farghal, A.I.A. [2] and Hassan, R.E. [1]

[1] *Plant Protection Dept., Fac. Agric., Al-Azhar Univ., Assiut, Egypt*

[2]*Plant Protection Dept., Fac. Agric., Assiut Univ., Egypt*

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Abstract:

The experiment was conducted to determine the efficacy of five inorganic salts viz., sodium chloride (NaCl), sodium fluoride (NaF), sodium phosphate (NaH_2PO_4), potassium chloride (KCl) and potassium phosphate (KH_2PO_4) against cowpea beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) . The effect of the salts was evaluated based on toxicity, oviposition, adult emergence and percentages of weight loss. After period 96 hrs, sodium fluoride showed the highest efficiency salt (LC_{50} 0.39 and LC_{90} 1.23%), followed by sodium chloride (0.42 and 2.33%) and potassium phosphate (0.43 and 1.39%). Sodium phosphate was the least toxic salt against the adult beetle (0.79 and 4.84%), whereas potassium chloride showed moderate toxicity (0.50 and 2.23%). The mean number of cowpea beetle eggs laid on cowpea seed treated with four concentrations of five inorganic salts, was significantly low as compared with control. Potassium phosphate and sodium fluoride affected oviposition of *C. maculatus* significantly. Results of adult emergence showed the same trend of oviposition, potassium phosphate and sodium fluoride showed high efficiency on adult emergence, sodium phosphate exhibited the lowest effective salt, whereas potassium chloride and sodium chloride were inbetween. Generally, based on direct toxicity, effect on oviposition, adult emergence and loss in seed weight, potassium phosphate and sodium fluoride showed great efficiency against the cowpea beetle, *C. maculatus*. So, it may be recommended to use these salts in control programme of this pest.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is a member of the family Fabaceae. It is a food and animal feed crop grown in the semi-arid tropics covering Africa, Asia, Europe, United States and Central and South America (Asante *et al.*, 2001), and also , a good source of energy (337.57-360.67 kcal / 100g), crude protein (25.79-29.25%), carbohydrate (53.56-57.36%), fat (0.79-3.18%), ash (2.72-3.73%) and crude fiber (1.92-3.37%), as well as small amounts of essential micronutrients including calcium, iron, magnesium and copper (Lambot, 2002 and Chinma *et al*., 2008). The cowpea beetle *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) has been recognized for years as the major insect pest of cowpea seeds (Ofuya, 2001; Ileke and Bulus, 2012 and Ileke *et al.,* 2013a). Huge losses of between 20 and 50% have been reported on stored cowpea due to the attack by cowpea beetle, *C. maculatus* and sometimes the loss could be complete accounting for 100% loss (Udo and Harry, 2013). Efficient control of stored products insect pests has long been the aim of entomologists throughout the world (Ileke *et al.,* 2013b). The control of stored products insects like *C. maculatus* has centered mainly on the use of synthetic insecticides (Asawalam *et al.*, 2007). However, the use of these chemicals is hampered by many attendant problems such as development of insect resistant strains, their toxic residues getting into food of animals and man, workers safety and high cost of procurement (Sighamony *et al.,* 1990 and Ileke and Oni, 2011). These problems have necessitated researcher to use an alternative eco-friendly cheaper means to control insect pests. It has been known for several years that some insects can be controlled by application of finely powdered substances, which are not chemically active. Desiccant dusts have been used traditionally as stored grain protectants. These dusts primarily exert their effects on insects through physical means. There are several group of desiccant dusts which can differentiated by their chemical composition or by their particle size (Golob, 1997 and Korunic, 1997 and 1998). The inert dusts have been used as a traditional method of insect control for thousands of years. The farmers in the developing countries had been used to mix the sand, wood ash, paddy husks etc in grains as grain protectants against stored grain insect pests. Therefore, based on degree of effectiveness against stored grain insect pests and chemical composition; these compounds can be categorized in to four groups. The first group mainly consists of the minerals such as lime (CaOH), lime stone ($CaCO_3$), salt (NaCl), dolomite, magnesite, copper, and Ketelsous (ground sulpher and rock phosphate). The sand, clay, kaolin (kaolinite, aluminum silicate hydroxide), paddy husks, wood ash and volcanic ash constitute the other group. The third group contains synthetic silica and the fourth one is of diatomaceous earth (Golob, 1997). Therefore, the objective of this study is to evaluate the efficiency of some inorganic salts on cowpea beetle.

Materials and methods

1. Rearing technique:

The beetles used in the present study were obtained from naturally infested cowpea seeds. Adult's *C. maculatus* were cultured in incubator at constant temperature of 27 ± 2°C and 70 ± 5% RH. in the Laboratory of the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Assiut Governorate. Uninfested cowpea seeds (*V. unguiculata*) were used for the experiment, purchased from a local market in Assuit city and disinfested in an oven at 60°C for 1 hour before using them as a substrate for insect rearing. Stock culture was set up by 50 pairs of *C. maculatus* introduced into the rearing bottles containing 250g seeds. The bottles were covered with muslin cloth and secured with rubber bands. The parent beetles were sieved out after 7 days of oviposition period. Later the seeds were kept in the incubator for adult emergence, which used for the experiment (Suleiman *et al.*, 2014).

2. Inorganic salts:

Inorganic salts used in the experiments were, sodium chloride (NaCl), sodium fluoride (NaF), sodium phosphate (NaH_2PO_4), potassium chloride (KCl) and potassium phosphate (KH_2PO_4). Each material was finely grounded in a porcelain mortar and passed through 0.1mm sieve. Four concentrations of each material (0.5, 1.0, 1.5 and 2.0%), were used.

3. Experimental set up:

To determine the effect of the experimental materials against *C. maculatus*. Quantities of 100g cowpea seeds were placed in glass jars 250 ml capacity and treated with an appropriate concentration of the experimental materials. The jars were shacked manually for a suitable time to ensure even coating of seeds, and then infested with 10 pairs of 1-2 day-old adult cowpea beetles *C. maculatus* for each jar. Each jar was covered with muslin cloth. Untreated cowpea seeds (control) were used as previously described. Three replicates were made for each treatment. Experiments were carried out under laboratory conditions.

3.1. For toxicity test:

In each treatment, observations were made and recorded for toxicity effect on mortality rates in all the jars after 24, 48, 72 and 96 hrs. The content of each jar was spread out in a try and the dead insects were removed and counted. After each count, the seeds and the alive insects were returned into the glass jars. The mortality in the control was also calculated. Percentage of mortality was corrected according to Abbott's formula (1925).

$$\% \text{ Corrected mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortaliy}}{100 - \% \text{ control mortality}} \times 100$$

3.2. Effect on the oviposition and progeny emergence:

After 14 days of infestation with the cowpea beetles, three replicates of random samples each containing 20 seeds of cowpea were removed from each jar and the number of eggs oviposited on them were counted and returned into the glass jars until progeny emergence. Progeny emergence in each replicate was taken for the first generation. At each time of observation, the newly emerged progenies were sieved out, counted and recorded.

3.3. Weight loss:

After 4 weeks of treatment, the weight loss of the seeds was evaluated by weighing the entire cowpea seeds in each jar and the difference from the initial weight of 100g was transformed into weight loss. According to the method of Mebarkia *et al.* (2010) used to calculate the loss in seeds:

$$WL = Wh - Wd$$

Where, WL = Weight loss.

Wh = Weight healthy seeds before infestation.

Wd = Weight damaged seeds after infestation.

4. Statistical analysis:

Data were analyzed using a one-way analysis of variance by MSTAT-C (1988) software package and means were separated using the least significant differences method only when a significant "F" test was obtained. Probit analysis was done to reckon either LC_{50} and LC_{90}, and confidence limits using SPSS V. 10 system software (SPSS Inc., 1999) and probit-line graphs were illustrated using Sigmaplot V. 8.02 Demo system software (SPSS Inc., 2002).

Results and discussion

1. Direct toxicity:

The LC_{50}, LC_{90} and their confidence limits, and slope value of LCP lines of five inorganic salts tested against *C. maculatus* for 96 hrs are shown in Figures (1-4).

After 24 hrs exposure (Figure,1) the least LC_{50} value was recorded for potassium phosphate (1.69%), whereas the values for the rest of inorganic salts were comparable. For LC_{90}, the sodium fluoride and chloride showed the least values (4.27 and 5.60%) but for the rest of inorganic salts, the values were comparable. According to the LC_{50} and LC_{90} values, sodium fluoride and chloride, and potassium phosphate showed relatively high efficiency against *C. maculatus* adults. Comparing the slope values, adult cowpea beetle showed relative high homogeneity response to sodium phosphate (3.89), sodium chloride (3.55) and potassium chloride (3.26). Data of 48 hrs exposure showed the same trend of 24 hrs results, but all values of LC_{50} and LC_{90} were less. Sodium fluoride and potassium phosphate showed the highest effective inorganic salts (LC_{50} 0.68 and 0.79%, and LC_{90} 3.14 and 3.41%, respectively). Sodium phosphate was the least effective one, LC_{50} and LC_{90} values were 1.78 and 5.78%. However, sodium chloride and potassium chloride showed moderate efficiency, the LC_{50} value was 1.45 and 1.47% and the LC_{90} value was 5.06 and 4.37%, respectively. Comparing slope values of LCP lines, cowpea adult showed relative homogeneity response to sodium phosphate (2.51) and sodium chloride (2.18) (Figure, 2).

The values of LC_{50} and LC_{90} of the organic salts after 72 hrs exposure, were less than that at 24 and 48 hrs exposure. Sodium fluoride exhibited the highest effective salt against cowpea beetle adult (LC_{50} 0.44 and LC_{90} 1.42%), followed by potassium phosphate (LC_{50} 0.53 and LC_{90} 1.97%), then sodium chloride (LC_{50} 0.59 and LC_{90} 3.87%), whereas sodium phosphate was the least effective salt (LC_{50} 1.32 and LC_{90} 5.35%). According to the slope values of LCP lines, adult beetle showed relative high homogeneity response to sodium fluoride (2.57) and potassium phosphate (2.25), the least homogeneity response was met with sodium chloride (1.58). However, potassium chloride and sodium phosphate were of moderate responses (2.09 and 2.11) (Figure, 3).

Data (Figure, 4) show the LC_{50} and LC_{90} values, and their confidence limits, slope value of LCP lines of five organic salts applied on cowpea beetle adult after 96 hrs. As in the previous exposure period, sodium fluoride showed the highest efficiency salt (LC_{50} 0.39 and LC_{90} 1.23%), followed by sodium chloride (0.42 and 2.33%) and potassium phosphate (0.43 and 1.39%). Sodium phosphate was the least active salt against the adult beetle (0.79 and 4.84%), whereas potassium chloride showed moderate toxicity against the adult beetle (0.50 and 2.23%). The cowpea adult beetle showed relatively high homogeneity response to sodium fluoride and potassium phosphate (slope value 2.57 and 2.52), whereas, the least homogeneity response was met with sodium phosphate (1.63) and sodium chloride (1.72).

2. Oviposition:

Data Table (1) represent the mean number of cowpea beetle eggs laid on cowpea seed treated with four concentrations of five inorganic salts, compared with control. Statistical analysis showed significant variation between treatments at all concentrations tested. Potassium phosphate showed the highest effective salt in reducing eggs laid, the mean number of eggs deposited on cowpea seeds at concentration 0.5%

was 5.66 eggs/20 seeds, decreased gradually as the concentration increased to attain 1.33 eggs at 2.0%. Sodium fluoride ranked second after potassium phosphate, the mean number of eggs laid cowpea seeds at 0.5% was 12.33 eggs decreased to attain 5.00 eggs at 2.0%. The lowest effective salt was sodium phosphate, at concentration of 0.5% the mean number of eggs laid on cowpea seeds was 27.67 eggs, decreased gradually by increasing concentration to attain 10.33 eggs at concentration 2.0%. Sodium chloride and potassium chloride showed moderate effect on ovipositon of *C. maculatus*. At 0.5% concentration, the mean number of eggs laid on cowpea seeds was 26.33 and 21.00 eggs, declined by increasing concentration to attain 2.66 and 9.33 eggs at 2.0%. The mean number of eggs laid on untreated cowpea seeds was 37.67 eggs.

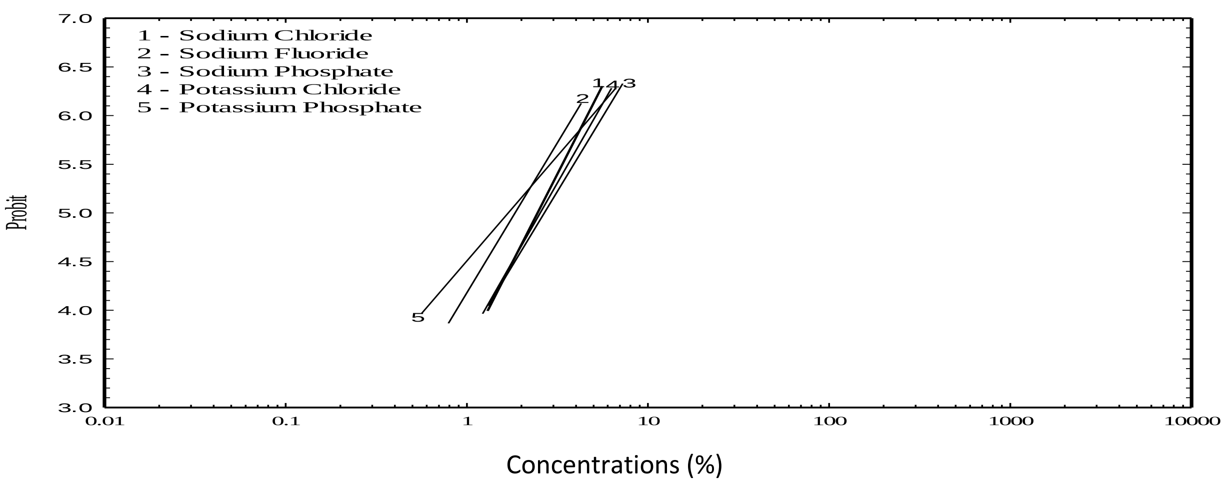

Figure (1): LCP lines of five-inorganic salts tested against C. maculatus at 24 hours

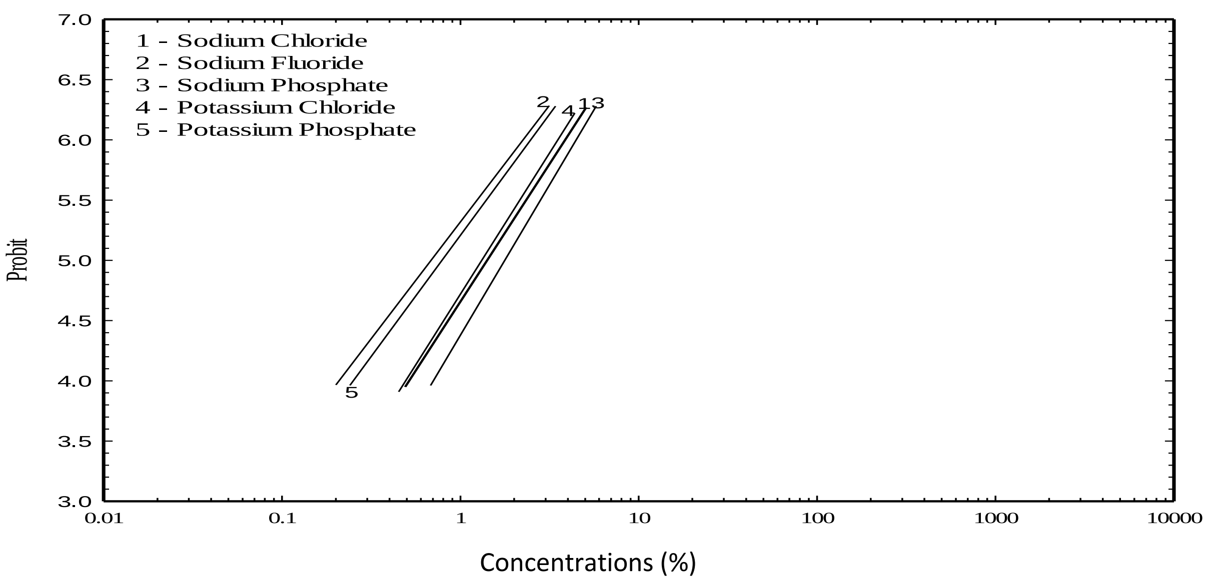

Figure (2): LCP lines of five-inorganic salts tested against *C. maculatus* at 48 hours.

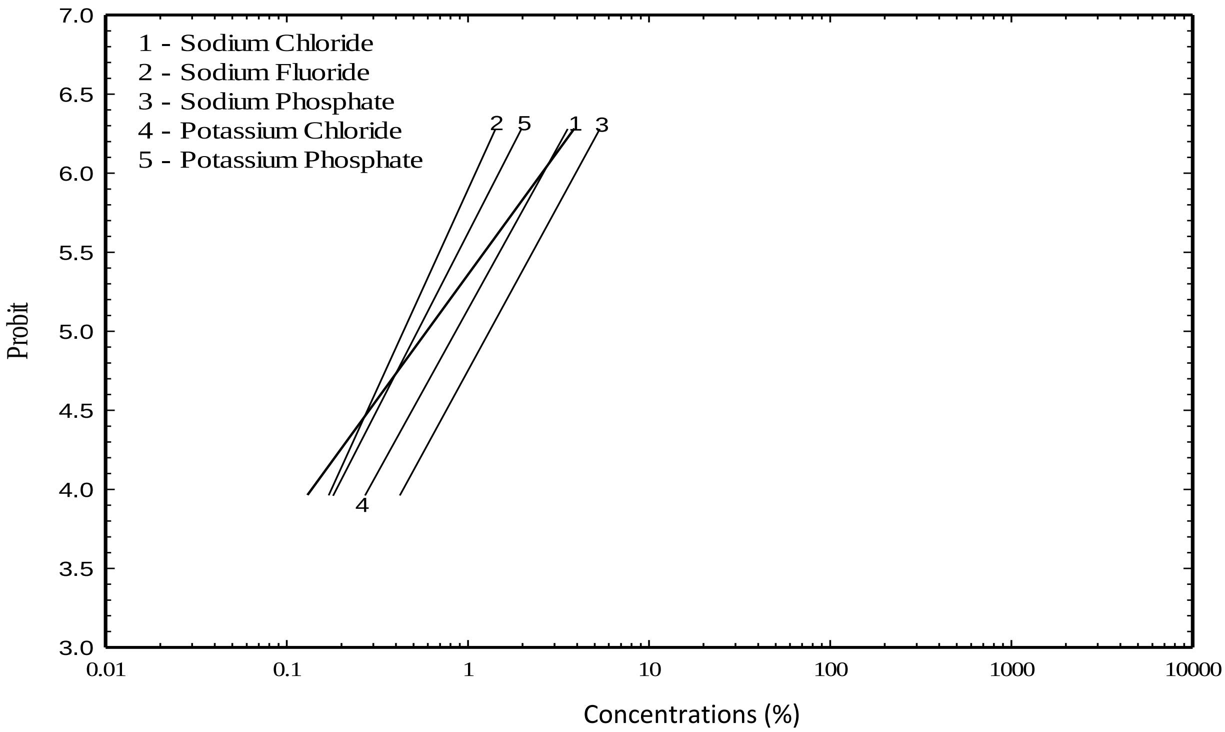

Figure (3): LCP lines of five-inorganic salts tested against *C. maculatus* at 72 hours.

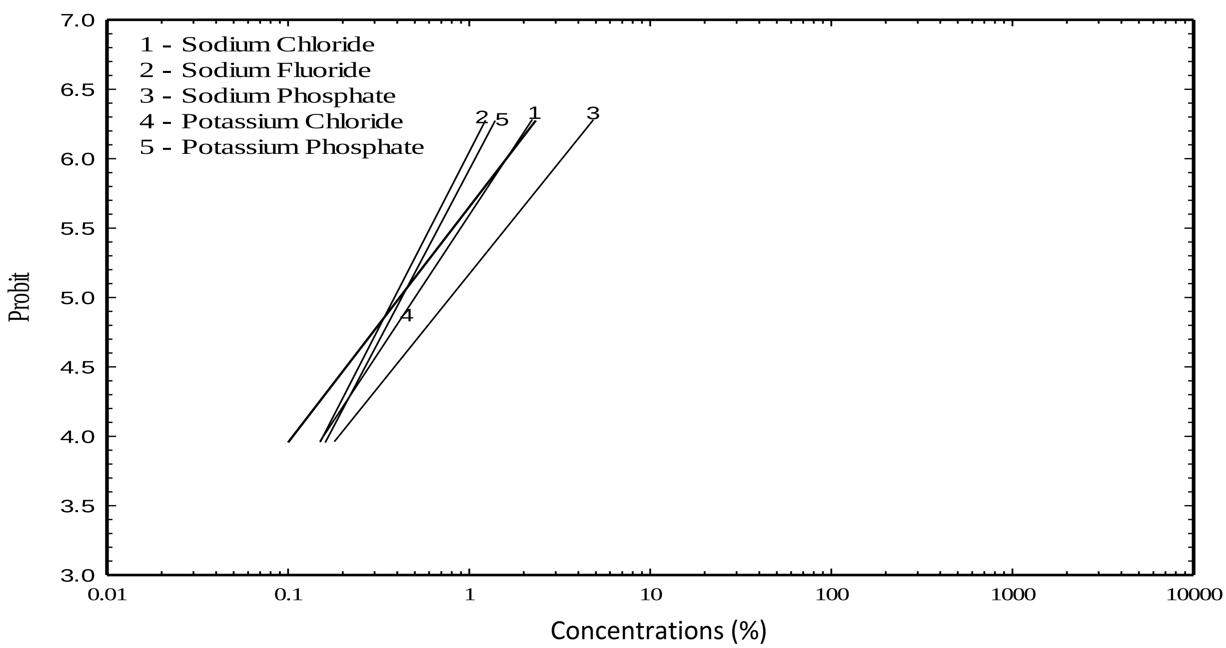

Figure (4): LCP lines of five-inorganic salts tested against *C. maculatus* at 96 hours.

3. Adult emergence:

Data Table (2) show the mean number of cowpea adults emerged from cowpea seeds treated with five inorganic salts compared with untreated seeds. In control, the mean number of cowpea adult emerged was 103.00 adult. Statistical analysis showed significant variation in adult emergence between treatments, and between concentrations. Regardless of concentrations applied, potassium phosphate and sodium fluoride exhibited the highest effective salts on adult emergence. At 0.5%, the mean number of adult emerged from cowpea seeds was 6.00 and 6.33 for the two salts respectively. The adult emergence decreased by increasing concentrations, no adult emerged at 1.5% of potassium phosphate, and at 2.0% sodium fluoride. The least efficient salt on adult emergence was sodium phosphate, at 0.5%, the mean number of cowpea adult emerged was 18.66, decreased gradually as concentration increased to attain 3.33 at 2.0%. However, potassium chloride and sodium chloride showed moderate efficiency against adult emergence, at 0.5% the mean number of adult emerged was 10.00 and 12.33, respectively, decreased gradually by increasing concentration to attain 1.66 and 0.00 at 2.0%.

4. Loss in seed weight:

Data Table (3) show the mean of loss in cowpea seed weight when treated with five inorganic salts at four concentrations compared with untreated seed. Results showed that, the mean of loss in seed weight in untreated seed was 4.84 g/100g seeds. Statistical analysis showed that treatment with inorganic salts significantly affect the seed weight loss. Potassium phosphate significantly reduced the seed weight loss, at 0.5% the mean of loss was 0.21g, declined to 0.10g at 1.0%, and no loss in seed weight was recorded at 1.5%. Sodium fluoride ranked second in reducing the mean of loss in seed weight at 0.5%, the average loss in seed weight was 0.39g decreased gradually by increasing concentration to attain 0.00g loss at 2.0%. Sodium phosphate showed the least salt in reducing seed weight loss, at 0.5% the loss was 1.15g to decrease gradually as concentrations increased to attain 0.09g at 2.0% concentration. However, potassium chloride and sodium chloride exhibited moderate effect on loss of cowpea seed weight. At concentration of 0.5% the two salts reduced the loss in seed weight by 0.62 and 0.91g. Whereas, at the highest concentration tested 2.0%, no loss in seed weight was detected for the two salts.

Table (1): Oviposition of *C. maculatus* on cowpea seeds treated with different concentrations of inorganic salts.

Concentration (%)	Mean no. of eggs /20 seeds ± SE				
	Inorganic salts				
	Sodium			Potassium	
	Chloride	Fluoride	Phosphate	Chloride	Phosphate
0.5	26.33 ± 0.88 A b	12.33 ± 0.66 C b	27.67 ± 1.20 A b	21.00 ± 1.15 B b	5.66 ± 1.20 D b
1.0	19.33 ± 1.45 B c	9.33 ± 0.66 C bc	24.33 ± 0.88 A bc	19.67 ± 0.88 B b	3.00 ± 0.57 D bc
1.5	10.33 ± 2.03 C d	6.66 ± 0.33 D cd	20.67 ± 0.33 A c	14.00 ± 0.57 B c	2.66 ± 0.66 E bc
2.0	2.66 ± 1.20 BC e	5.00 ± 0.57 B d	10.33 ± 1.45 A d	9.33 ± 1.20 A d	1.33 ± 0.88 C c
Control	37.67 ± 2.18 a	37.67 ± 2.18 a	37.67 ± 2.18 a	37.67 ± 2.18 a	37.67 ± 2.18 a

Means, in the same column / row, followed by the same letter are not significantly different at 0.05 level of probability.

- Small letter for concentrations (columns)
- Caps letter for treatments (rows)

Table (2):Adult emergence of *C. maculatus* from cowpea seeds treated with different concentrations of inorganic salts.

Concentration (%)	Mean no. of adult emerged in F_1 ± SE				
	Inorganic salts				
	Sodium			Potassium	
	Chloride	Fluoride	Phosphate	Chloride	Phosphate
0.5	12.33 ± 1.20 B b	6.33 ± 0.88 C b	18.66 ± 1.45 A b	10.00 ± 0.57 B b	6.00 ± 0.57 C b
1.0	7.00 ± 0.57 B bc	4.00 ± 0.57 CD bc	11.33 ± 0.88 A c	6.33 ± 1.45 BC bc	2.66 ± 0.33 D c
1.5	4.66 ± 0.88 AB cd	0.33 ± 0.33 C c	6.66 ± 1.20 A cd	3.00 ± 1.00 B c	0.00 ± 0.00 C c
2.0	0.00 ± 0.00 C d	0.00 ± 0.00 C c	3.33 ± 0.33 A d	1.66 ± 0.33 B c	0.00 ± 0.00 C c
Control	103.00 ± 4.04 a	103.00 ± 4.04 a	103.00 ± 4.04 a	103.00 ± 4.04 a	103.00 ± 4.04 a

Means, in the same column / row, followed by the same letter are not significantly different at 0.05 level of probability.

- Small letter for concentrations (columns)
  - Caps letter for treatments (rows)

Table (3): Loss of seed weight cowpea treated with different concentrations of inorganic salts due to *C. maculatus*.

Concentration (%)	Mean (g/100g seeds) ± SE				
	Inorganic salts				
	Sodium			Potassium	
	Chloride	Fluoride	Phosphate	Chloride	Phosphate
0.5	0.91 ± 0.06 B b	0.39 ± 0.06 CD b	1.15 ± 0.07 A b	0.62 ± 0.07 C b	0.21 ± 0.03 D b
1.0	0.36 ± 0.03 B c	0.20 ± 0.02 C c	0.80 ± 0.03 A c	0.44 ± 0.05 B c	0.10 ± 0.02 C c
1.5	0.17 ± 0.05 B d	0.07 ± 0.01 BC d	0.51 ± 0.05 A d	0.11 ± 0.02 BC d	0.00 ± 0.00 C d
2.0	0.00 ± 0.00 A e	0.00 ± 0.00 A d	0.09 ± 0.06 A e	0.00 ± 0.00 A e	0.00 ± 0.00 A d
Control	4.84 ± 0.10 a	4.84 ± 0.10 a	4.84 ± 0.10 a	4.84 ± 0.10 a	4.84 ± 0.10 a

Means, in the same column / row, followed by the same letter are not significantly different at 0.05 level of probability.

- Small letter for concentrations (columns)
- Caps letter for treatments (rows)

The toxic effects of calcium phosphate have been reported by Majumder and Bano (1964). The use of mineral salts offered a new promising method of insect control (Pratt *et al*., 1972). The salt tri-calcium phosphate ($Ca_3 PO_4)_2$ or TCP) has shown promise as insect population suppressant when added to blended cereal foods. In Egypt, El-Halfawy (1977) showed that hydrated lime had the greatest inhibitory effect on the bruchid. He tested the mixing dusts of twelve inert materials with cowpea (*V. unguiculata*) at a concentration of 1.0% on the adult life-span and productivity of *Callosobruchus chinensis* L. Ignatowicz and Boczek (1978) found that iodine salts induced sterility in *Tyrophagus putrescentiae* (Schra.) by reduction of egg production rather than by reduction of their hatchability. They added that females were more susceptible than males because iodine salts exerted detrimental effect upon formation of eggs. Hassan (1981) found that NH_4NO_4 and Ca_3 $(PO_4)^2$ caused the strongest inhibition of *Batrachoides surinamensis* (Bloh and Schneider) development while KH_2PO_4 and $(NH_4)3PO_4.3H_2O$ proved to be the most stimulatory salts for this species. Davis *et al*. (1984) reported on tri-calcium phosphate (TCP) as a legume grain protectant against three bean weevils where mortality was recorded as occurring within 8 hrs. TCP at 0.1 and 0.25% by weight dusted on navy bans or cowpeas as a protectant prevented the occurrence of a F_1 generation. Research on inorganic salts as grain protectants has been conducted at the USDA-ARS (Highland *et al*., 1984 and Bookwalter *et al*., 1985). Le-Patourel (1986) assessed the toxicity of a sorptive silica dust in samples of wheat to adult populations of *S. granarius*, *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.) at grain moisture contents between 9.4 and 18.7%. The tolerance of these species to the dust treatments was found to increase with increasing moisture content and to be unrelated to their relative abilities to survive short periods at low relative humidites when provided with food. The amorphous silica dust

(Dryacide) was used by Aldryhim (1990) to treat wheat grains at concentrations of 0.0, 250, 500, 750, and 1000 ug silica dust /kg wheat. Adults of *S. granarius* and *Tribolium confusum* Duv. were placed in the grains which were then incubated at 20 or 30°C and 40 or 60% R.H. Mortality counts were taken after 48 and 168 hrs. *S. granarius* was more susceptible to silica dust than *T. confusum* under the same conditions. Silica dust reduced progeny by 100% at 40% R.H. at all used concentrations. Progeny were produced by *S. granarius* at 30°C and 60% R.H. but with significantly reduced numbers by increasing dosage. In recent study Mahmoud (2012) found that silica dust and tri-calcium phosphate had high repellent effect on the granary weevil, *Sitophilus granarius* L., at concentration of 5.0 g/kg grains.

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