Assiut Journal of Agricultural Sciences 54 (3) 2023 (53-64)
ISSN: 1110-0486 / EISSN: 2356-9840
Website: http://ajas.journals.ekb.eg/
E-mail: ajas@aun.edu.eg

(Original Article)

Efficacy of Certain Natural Compounds and Entomopathogenic Fungi Compared with Malathion Against Cowpea beetle, *Callosobruchus maculatus* (Fabricius) under Laboratory Conditions

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DOI: 10.21608/AJAS.2023.199238.1240
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Abstract

Cowpea beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) is one of the most dangerous insect pests in field and storage. It causes a high damage to the legumes by decreased dietary values, germination and seed weights. Using synthetic insecticides for controlling stored grain pests have a lot of problems for humans and environment. Therefore, the mixing seeds laboratory experiments were conducted to study the efficacy of certain alternative safety compounds against *C. maculatus* such as *Beauveria bassiana*, *Metarhizium anisopliae*, ascorbic acid, boric acid, talc powder and kaolin compared with an organophosphorus nervous insecticide, malathion 1% dust, that recommended in Egypt for controlling stored grain pests. The present results showed that the malathion was the highest toxicity effect against cowpea beetle adults with LC_{50}(3.89 ppm) after 3-day post treatment. However, the boric acid was the lowest toxicity effect with LC_{50} (72855 ppm) after 6 days post treatment. The other tested materials showed moderate effects. These results indicate that malathion is still having high effect against cowpea beetle. The toxicity of bio-insecticides (*M. anisopliae* and *B. bassiana*) after 6days were more than toxicity malathion 1%dust after 1day.Wheraes, the toxicity of bio-insecticides (*M. anisopliae* and *B. bassiana*) increased with increase of period of exposure from 2 to 6 days high rates about 28 times, 31 times, respectively. The findings showed that the tested compounds have a promising insecticidal activity against *C. maculatus* and can be used as possible alternatives to synthetic chemical insecticides such as malathion for the control of stored product insects.

Keywords: *Callosobruchus maculatus,* Malathion, Entomopathogenic fungi.

Introduction

Crop legumes cultivated in cold or temperate climates are exposed to insects that are serious global pests of legumes because they cause severe damages when they feed directly, additionally since they transmit dangerous bacteria and fungi(Qaim and Zilberman, 2003; Hubert *et al.*, 2004; Zayed, 2012; Heie, 2013). In Egypt cowpea is a significant legume crop and a major source of protein, it is used of dry seeds, fodder and green pods as well as it is tolerant drought, heat and

Received: 13 March 2023/ Accepted: 26 June 2023/ Published online: 1 July 2023

improving soil fertility. The total area under cultivated of cowpea plant in Egypt was estimated at 1853 ha with a mean production of 7180 tons of dry seeds (Elsobky and Hassan, 2021). Three major groups of cowpea pests can be identified: preflowering, flowering/postflowering, and storage. Some of these pests are: cowpea aphid, *Aphis craccivora* ; flower thrips, *Megalurothrips sjostedti* ; blister beetles, *Mylabris* spp.; maruca pod borer, *Maruca testulalis*; pod-sucking bugs, *Anoplocnemis curvipes* and cowpea beetle, *Callosobruchus maculatus* (Fabricius), (Coleoptera:Bruchidea) (Dugje *et al.*, 2009). Cowpea beetle is one of the most economic insect pests of stored legume seeds that causes losses in essential legumes up to 50% such as faba bean, field pea, chickpea and lentil (Ali and Habtewold, 1993; Telaye *et al.*, 1994; Damte and Dawd, 2003; Ali *et al.*, 2006).When the seed moisture content is high , damage starts in the field and reaches to the storage (Ntoukam *et al.*, 2000). If insect pests are not successfully controlled, the damage in cowpea seeds will reach 80-100% (Dugje *et al.*, 2009). One of the popular methods used to control cowpea beetles are synthetic insecticides especially methyl bromide and phosphine as fumigants. Although they are high effective , and have several side effects such as human health risks , environmental contamination (ozone depletion) , adverse effects on non-target organisms and pest resistance (Hansen and Jensen, 2002; Whitford, 2002; Benhalima *et al.*, 2004; Bughio and Wilkins, 2004; Boyer *et al.*, 2012). Therefore, there is rapid necessity to find suitable approaches depending on bio-rational materials for control of stored-grain pests(Sukumar *et al.*, 1991) that have rather safe , less persistence , cheap and ecofriendly (Magaji *et al.*, 2005). Among of those material, *Beauveria bassiana* (Abdu-Allah *et al.*, 2015; Batta and Kavallieratos, 2018) , *Metarhizium anisopliae* (Ozdemir *et al.*, 2020; Iqbal *et al.*, 2021) , ascorbic acid (Garg and Mahajan, 1994; Ahmad *et al.*, 2013) , boric acid (Kausar *et al.*, 2012; AS and Azher, 2020) , talc powder (Ciobanu and Drosu, 2009; Alice and Srikanth, 2013) , kaolin (Kéita *et al.*, 2001; Gad *et al.*, 2022) , malathion 1% dust (Gharib, 2006; Zayed, 2012) . Re-evaluation of the effectiveness of the bio-rational materials against cowpea beetle adults compared with the recommended organophosphate insecticide, malathion 1% dust should be investigated. To find the alternative material could be replaced malathion or could be used in pest management programs of cowpea beetle/stored grain insect pests.

Materials and Methods

Insect

According to Suleiman *et al.* (2014), Cowpea beetle , *C. maculatus* adults were obtained from the Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC),Egypt. The insects were reared on cleaned uninfested cowpea and they were placed inside glass containers with cover perforated and covered with a muslin cloth to prevent the escape of the insects, prevent other intruders, and to allow for ventilation. These containers were placed under laboratory conditions 25±2C° and 65±5% (R.H.) in the Laboratory of Plant Protection Department, Faculty of Agriculture, Assiut University.

Compounds used

Two entomopathogenic fungi products produced by (PPRI), (ARC), Egypt; *Beauveria bassiana* (Balsamo) Vuillemin and (Biover®, 10 % WP) and *Metarhizium anisopliae* (Metchnikoff) (Bioranza®, 10 % WP). L-Ascorbic acid (ascorbic acid®), Hydrogen borate (boric acid®) were purchased from Egyptian El-Gomhouria Company. Hydrous magnesium silicate (talc powder®), Hydrated aluminum silicate (kaolin®), and malathion dust (malason®, 1% dust, Kafr El-Zayat Pesticides and Chemicals) were purchased from local markets.

Mixing seeds bioassay

According to Zayed (2012) with some modifications to test the potency of the adults of *C. maculatus* under controlled laboratory conditions. To every tested compound,5-7 concentrations were prepared, diluted by wheat flour to be a final 2gram volume dust. Five grams of cowpea seeds mixed with the 2gram volume. Ten unsexed adults (0-48h old) of *C. maculatus* were exposed to the treated seeds in Petri-dishes (9 cm diameter). Three replicates of each treatment were prepared. A similar treatment with flour alone was used as control. The experiments were duplicated to every compound. The treatments were incubated in 3 days in petri dishes under laboratory conditions 25±2C°and 65±5% (R.H.). The adult mortality data were recorded after 1,2,3,4,5,6 days post-treatment. If a brush was used to gently push a cowpea beetle's abdomen and there was no response, the insect was confirmed dead. Mortality percentages were corrected by Abbot's formula(Abbott, 1925). The LC_{50s}, slope, toxicity index values were calculated by SPSS. The same software was used to estimate the LC_{50}, the 95% confidence limit (CL) values, the slope, and the x^2. LC_{50} values were considered significantly different when their 95% CLs did not overlap (Schenker and Gentleman, 2001). Log concentration versus percent mortality (probit) regression lines were generated to determine the Compounds concentration that killed 50% of the adults (LC_{50}) from each treatment.

Results

Data in Table (1) and Figure (1) showed the LC_{50}, slope values of the two bioagent (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc and kaolin powders) compared with malathion insecticide against *C. maculatus* adults (0 – 48 h old) using mixing seeds methods. Based on the LC_{50} values, the toxicity of tested compounds after 3 days post treatment could be ascending as: malathion > *B. bassiana* > *M. anisopliae* > kaolin > talc powder. Although, the low toxicity index values of the tested compounds (0.04-0.97) compared (100.00) in malathion after 3 days from treatment, and the adult mortalities were increased with the increase of period of exposure especially with the tested two bioagents. The toxicity of bio-insecticides (*M. anisopliae* and *B. bassiana*) after 6 days was more than toxicity the phosphorous pesticide, malathion after 1day.The toxicity of bio-insecticides (*M. anisopliae* and *B. bassiana*) increased with increase of period of exposure from 2 to 6 days, whereas the toxicity values increased by 28 and 31 times, respectively The results demonstrated that malathion was the most effective agent against *C.*

maculatus followed by *M. anisopliae* and *B. bassiana*, respectively. After 6 days of exposure, the LC_{50} values of *B. bassiana*, *M. anisopliae* and talc powder recorded 232.11, 217.15 and 1359.57 ppm, respectively. After 1- and 2-days exposure; the LC_{50} values of kaolin and malathion were 45072.93, 12532.53 and 439.29, 9.42 ppm, respectively. Based on the LC_{50} values, malathion demonstrated that the highest toxic insecticide against cowpea beetle after 1 and 3 days 439.29, 9.42,3.89 ppm, respectively.

Table 1. Efficacy of two bio-agents (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc powder and kaolin powder) compared with malathion dust against *C. maculatus* adults (0 – 48 h old) using mixing seeds method after 1,2,3,4,5,6 days from treatment.

Toxicity Compounds	Time(day)	LC_{50} (ppm) (C. Ls95%)	Slope±SE	x^2	Sig.	Toxicity Index (TI)
B. bassiana	3 days	6562.32 c (5514.02-7921.55)	1.58±0.15	4.04	0.26	0.06
	4 days	2284.66 d (1948.06-2668.44)	1.82±0.16	3.23	0.36	-
	5 days	763.87 b (602.11-939.72)	1.49±0.13	2.92	0.4	-
	6 days	232.11 b (120.23-318.13)	1.66± 0.34	0.72	0.39	-
M. anisopliae	3 days	6796.91 c (5754.29-8142.82)	1.67±0.16	3.03	0.39	0.06
	4 days	1766.56 d (1495.59-2120.29)	1.68±0.16	5.09	0.17	-
	5 days	513.23 b (378.42-647.17)	1.34±0.15	4.66	0.19	-
	6 days	217.15 b (117.29-294.89)	1.86±0.36	0.31	0.958	-
Talc powder	2 days	12209.76 c (5047.54-20022.55)	1.08±0.17	1.88	0.59	0.08
	3 days	7020.99 c (3687.02-10412.65)	1.51±0.19	1.87	0.6	0.05
	4 days	5334.37 c (3784.49-6950.72)	1.29±0.3	0.76	0.38	-
	5 days	4145.38 c (3108.79-5040.81)	1.83±0.32	0.32	0.57	-
	6 days	1359.57 b (438.65-2041.28)	2.65±0.67	1.09	0.29	-
Kaolin	1 day	45072.93 e (21571.92-85241.69)	1.99±0.16	16.25	0.001	0.97
	2 days	12532.53 c (3646.52-20118.87)	2.04±0.23	7.89	0.05	0.08
	3 days	5141.88 c (3947.78 – 6326.19)	1.53±0.16	1.44	0.69	0.08
Malathion dust	1 day	439.29 b (269.43-835.03)	0.64±0.07	0.53	0.91	100
	2 days	9.42 a (3.14-17.34)	0.43±0.1	0.39	0.94	100
	3 days	3.89 a (0.87-8.26)	0.65±0.12	0.99	0.81	100

[TI] Toxicity Index = (LC_{50} value of most toxic compound (malathion dust) / LC_{50} value of tested compound) X 100; a,b,c,d,e LC_{50} values marked with different lower-case letters are significantly different based on non-overlap of 95% confidence limits

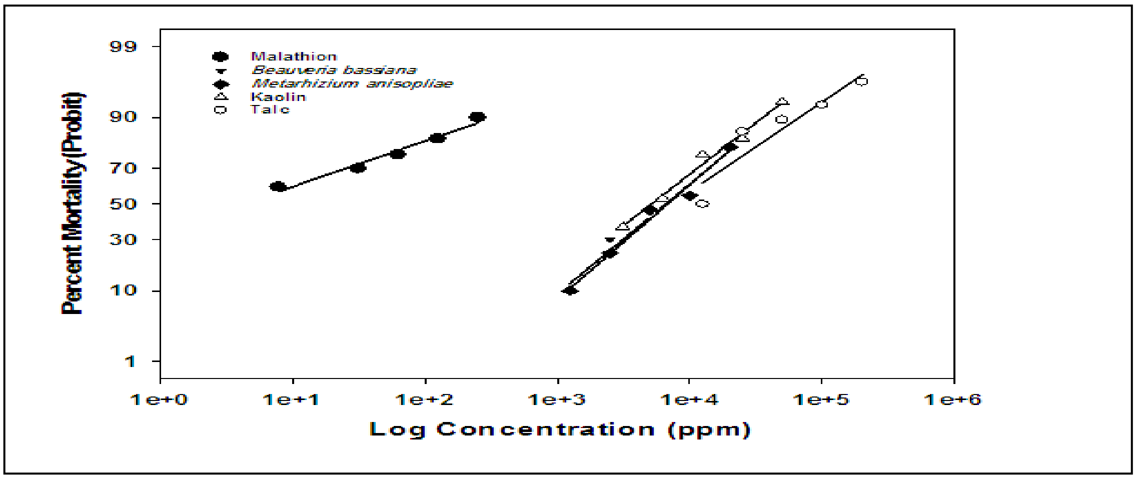

Figure 1. concentration response curves for five compounds against adults of cowea weevil, *Callosobruchus maculatus*. Adults (0-2 days old) were exposed to different concentrates and mortality was determined 72 h after exposure. Data analyzed using probit analysis in SPSS (Chicago, USA). For each dose, 3-4 replicates were done.

On the other hand, kaolin was the lowest effective one after 3 days with LC_{50} 5141.88 ppm. The toxicity lines were sharper with malathion dust, *M. anisopliae* and *B. bassiana.* While the high slope value was recorded for talc powder against cowpea beetle (2.65) after 6 days, the least one with malathion dust (0.43) after 2 days. The LC_{50} values of tested materials decreased by increasing exposure time. The data is represented in Figure (1) the high slope value was recorded for malathion.

Data in Table 2. showed after 6 days of exposure, the LC50 values of Ascorbic acid and Boric acid were 32708.56 and 72855.29 ppm, respectively.

Table 2. The efficacy of two antioxidants (ascorbic and boric acids) against *C. maculatus* adults (0 – 48 h old) using mixing seeds method after 5 and 6 days from treatment.

Toxicity against *C. maculatus*						
Toxicity Compounds	**Time(day)**	**LC_{50} (ppm) (C. Ls95%)**	**Slope±SE**	**x^2**	**Sig.**	**Toxicity index** (TI)
Ascorbic acid	5days	8.291E5 (406193.24-4506451.32)	0.64±0.14	0.75	0.86	42.6
	6 days	32708.56 (13318.48-52194.02)	0.65±0.14	0.45	0.93	100
Boric acid	5days	3.532E5 (229390.59-762597.89)	0.78±0.14	1.44	0.69	100
	6 days	72855.29 (57063.19-90385.59)	1.24±0.14	2.34	0.51	44.89

TI Toxicity Index = (LC_{50} value of most toxic compound / LC_{50} value of tested compound) X 100; (-) Undetected

Discussion

Our results showed that the malathion was the most potent agent against cowpea beetle adults. These result supported by Zayed (2012), who found that malathion is the most efficient among plant materials mustard, anise, black pepper seeds and turmeric rhizomes by mixing with wheat grains. The author stated that malathion is the inhibition of acetylcholinesterase enzyme activity, causing nervous and respiratory damages that may potentially result in death.

In the present study, two tested entomopathogenic fungi showed mortality rates after 3 days of treatment against cowpea beetles, LC_{50} value of *M. anisopliae* was lower than *B. bassiana* until 6 days. So that, *M. anisopliae* was more toxic than *B. bassiana*. Results of this research were similar to findings of Abdu-Allah *et al*. (2015), where *M. anisopliae* was more efficient than *B. bassiana* against *C. maculatus*. Lawrence and Khan (2002) reported that *M. anisopliae* was more virulent than *B. bassiana* against *C. maculatus*. *M. anisopliae* showed 92.50%, *B. bassiana* 87.50% mortality of *C. chinensis* by (Khan *et al*., 2018). *M. anisopliae* was proved the most effective virulent resulting in 98.33% mortality in the application of *B. bassiana* of Khapra beetle, *Trogoderma granarium* (Everts) by Iqbal *et al*. (2021). Though certain species can also infect the digestive tract, fungus primarily infect their hosts through the exterior insect cuticle. The fungus develops as hyphal bodies once it enters the hemolymph. In most cases, nutritional loss, organ infestation, and the activity of fungal toxins combine to cause host death by Inglis *et al*., (2001).

Kaolin is a clay mineral that occurs naturally and is chemically inert. It has been applied to reduce the damaging effects of environmental stress on crop plants and to protect crops from insect pests by Mahmoud *et al*. (2010) . Our results showed that, kaolin at 5000 mg/kg caused approximately 50% mortality rates of cowpea beetles, after 3 days. While in treatment with Kaolin at 1000 mg/kg caused the highest insecticidal activity with adult mortality of 73.7 and 80.9% for *C. maculatus* after 3 and 7 days from treatment. kaolin at 1000 mg/kg after 3 days, it caused the mortality 59.9 and 72.4 % of *C. chinensis* adults by Gad *et al*. (2022). The variation in the results could be explained by the kaolin purity used and the bioassay modification, where the cowpea beetles have higher contact than in our bioassay. Moreover, it may be the variant in cowpea variety.

Our results showed low effect of talc against the cowpea beetles. The result agreed with Ciobanu and Drosu (2009) who found that talc powder used as grain protectants against stored grain insects, it caused promoting moisture evaporation and death by desiccation (Alexander *et al*., 1944). Talc recorded inefficient results for the protection of the stored bean seeds against bean weevil *Acanthoscelides obtectus*, the population of the pest recovering after 50 days from treatment, the same as in control variant by. On the other hand, Yadav and Tiwari (2017) found that the inert materials such as talc powder was effective against adult emergence, reduced damage and weight loss of *Sitophilus oryzae* and *Rhyzopertha dominica* on treated wheat grains.

Antioxidants can fight off free radicals and prevent us from variety of diseases. They either remove reactive oxygen compounds. Such as ascorbic acid (vitamin C) is responsible of preventing or reducing the negative effects of oxidative stress (Ahmad *et al*., 2013).our results showed that the LC_{50} values of ascorbic acid on *C. maculatus* were low until 6 days due to antioxidants, ascorbic acid increased the life span of *C. maculatus* by decreasing age-independent susceptibility to death by Garg and Mahajan (1993).

Boric acid is a non-volatile mineral with insecticidal, fungicidal, and herbicidal effects that is not harmful to mammals. Insects are killed by drying out after absorbing boric acid chemicals through the wax layer of their outer surface(Woods, 1994). (AS and Azher, 2020)conducted effects mixture of powder (boric acid/silica) by mixing with the wheat seeds on the khapra beetle, *Trogoderma granarium* and studied adding inert powders on the surface of stored grain to reduce insect infestation by dusting grain that has been stored with inert powders. *Trogoderma granarium* and demonstrate the effectiveness of natural and synthetic inert powders to control larval and adult stages, for exposure periods of 4 and 6 days, and that the adults were more sensitive to the inert substance by twice compared to the third-age larvae. (Sahoo and Sahoo, 2017) studied the milled rice grains were mixed with doses (0.1% and 0.15%) of inorganic inert materials (the diatomaceous earth, camphor and boric acid).Ten insects of rice weevil were exposed to treated rice. The results showed that boric acid treatment was the lowest LC50 value 0.01% at 72 hours after treatment against rice weevil, *Sitophilus oryzae*.

The slope values were1.08 to 2.65 for tested materials, these indicated that population of cowpea beetle was homogeneous with tested materials compared with malathion dust. The response of population to malathion may be less homogeneous.

Based on the overlapped of confidence limits rates, there are significant difference in LC_{50} values between malathion and rest tested compounds. Also, there are significant differences between *B. bassiana* and *M. anisopliae* LC_{50} values of 3 and 4 days. There are significant between talc powder after 6 days with 2,3,4,5 days. There are significant between kaolin after 1 day with 2,3 days.

In conclusion, the toxicity of bio-insecticides (*M. anisopliae*, *B. bassiana*) after 5 days post treatment was as similar as toxicity of the phosphorous pesticide, malathion after 1 day. The toxicity of bio-insecticides of (*M. anisopliae* and *B. bassiana*) after 6 days was more than toxicity the phosphorous pesticide, malathion after 1 day. The toxicity of bio-insecticides (*M. anisopliae*, *B. bassiana*) increased with increase of period of exposure from 2 to 6 days high rates about 28 times and 31 times, respectively. The toxicity of natural compounds (talc powder and kaolin) increased with increase of period of exposure, the increase is not high reached from 2 to 6 days about 9 times.

Acknowledgment

The authors introduced great appreciation for Plant Protection Department, Fac. Agric. Assiut University for partial funding.

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فعالية بعض المركبات الطبيعية والفطريات الممرضة للحشرات مقارنة بالملاثيون ضد خنفساء اللوبيا تحت الظروف المعملية

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الملخص

تعد حشــرة خنفســاء اللوبيا واحدة من أخطر الآفات الحشــرية في الحقل والمخزن. تســبب الحشــرات ضــررًا كبيرًا للبقوليات حيث تقلل القيمة الغذائية وتقلل نسـبة الانبات وتسـبب فقد في محصـول البذور. تسـتخدم المبيدات الحشـرية الصـناعية لمكافحة افات الحبوب المخزونة وتسـبب العديد من المشاكل للإنسان والبيئة. لذلك أجريت تجارب خلط البذور المعملية لدراسة فعالية بعض المركبات كبدائل آمنة ضد الحشرات الكاملة لخنفساء اللوبيا. وهذه المركبات هي فطري البيوفاريا، فطر الميتارهيزم، حمض الاســـكوربيك، حمض البوريك، بودرة التلك والكاولين مقارنة بالمبيد الفوســـفوري العصـــبي (الملاثيون) والموصـــي به في مصـــر لمكافحة افات الحبوب المخزونة. أظهرت نتائج الدراسـة المعملية ان مبيد الملاثيون له فعالية عالية ضـد خنفسـاء اللوبيا وكانت قيمة التركيز الســـام النصـــفي هو3.89 جزء في المليون بعد 3 أيام من المعاملة. بينما حمض البوريك كان له اقل سمية وكانت قيمة التركيز السـام النصـفي بعد 6 أيام من المعاملة هو 72855. أما بقية المواد المختبرة الآخرى لها تأثير متوسط على الحشـرات المختبرة. لذلك توصـي النتائج باسـتخدام المبيد الحشـري الفوسـفوري العضـوي الملاثيون حيث أن له تأثير عالي ضـد آفات المخازن. بينما المبيدين الحيويين (البوفاريا باسانيا والميتارهيزم أنيسوبلاي) ازدادت فعاليتها مع زيادة الوقت بعد المعاملة. حيث أن فعالية البيوفاريا والميتارهيزم بعد 6 أيام كانت أكثر من المبيد الفوســـفوري الملاثيون بعد 1 يوم من المعـاملـة. الفعـاليـة للمبيـدين الحييوين البيوفـاريـا والميتـارهيزم تزداد مع زيادة الوقت بزيادة عالية جدا تصـــل الي 28 ضـــعف، 31 ضـــعف، على التتابع. بعض البدائل المختبرة واعدة في استخدامها في مكافحة هذه الآفة.

كلمات افتتاحية: خنفساء اللوبيا، مبيد الملاثيون، الفطريات الممرضة للحشرات.