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(Original Article)

The Effectiveness of Certain Insecticides and Combined Activities Against Adult Cowpea Beetles

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Abstract

Callosobruchus maculatus is a common pest in legume fruits. Herein, this study was conducted to evaluate the toxic potential of certain chemicals against cowpea beetle using filter paper and dipping seeds bioassays. As a result, carbosulfan, a carbamate insecticide, was the most toxic, followed by indoxacarb and malathion. The LC_{50} values varied in both tested bioassays. In general, in filter paper contact and dipping seeds as residual bioassay, all tested insecticides categories have the same toxicity ranking. Whilst neuro insecticides have the first ranks, the IGRs have the later ranks.

These results suggest certain tested insecticide is more suitable in contact, while others are more effective in mixing with seeds such as thiamethoxam. While carbosulfan and malathion are good candidates as store or seed protectant agents. Also, the emamectin benzoate and spinosad are promised ecofriendly agent as contact and residual effects as protectant against storage insect pest. Neuro insecticides were the highest toxicity among the tested insecticides, while IGRs have the lowest. Some insecticides are more suitable for contact, while others are more effective in mixing with seeds. Emamectin benzoate and spinosad are eco-friendly agents for contact and residual effects. Synergistic activity was detected in thiamethoxam, lemongrass oil, jojoba oil, and flaxseed oil.

Keywords: *Cowpea beetle, Synergistic activity, Pesticides*

Introduction

Callosobruchus maculatus (F.) is considered one of the most important insect pests of legumes in fields and stores. Due to their fast development, cowpea weevils can severely damage every seed that is being stored, resulting in weight losses of up to 60% (Keita *et al*., 2000). To avoid some losses throughout the storage period, use of pesticides is very limited due to their serious impact on humans, foods, and environment, beside insecticide resistance (Pacheco *et al*., 1990). Screening the toxicity of different insecticides from different groups that have different mode of action is essential in integrated pest management program. Carbosulfan, a carbamate insecticide, inhibits the acetylcholine esterases in the nervous system that is responsible for catalyzing the acetylcholine

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(neurotransmitter compound) to acetic acid and choline (Fukuto,1990). Cypermethrin, a synthetic pyrethroid insecticide, neuron toxic, knockdown effect, one of the safest synthetic insecticides. It acts through block voltage sodium channel in nervous system (Field *et al*., 2017). Neonicotinoid insecticides act as nicotinic acetylcholine receptors (nAChRs) (Taillebois *et al*., 2018). They are commonly used in agricultural pest programs. Insect growth reg against *C. maculatus* adults ulators (Gad *et al*., 2021). The use of plant essential oils alone was less effective than commercial insecticides and the possibility of using these oils in combination with synthetic insecticides in simple mixture seem attractive and effective (Don Pedro, 1989a and 1989b). One solution that integrated pest management (IPM) programs have implemented is the use of synergists in combination with insecticides that have various modes of action (Ishak *et al*., 2015). Piperonyl butoxide (PBO) is a potent synergist that inhibits cytochrome P450 monooxygenases' ability to detoxify insecticides and lowers insecticide resistance in *Aedes aegypti* (Paul *et al.,* 2006). The current study aimed to evaluate the efficacy of certain synthetic insecticides against *C. maculatus* adults using filter paper and treated seeds bioassays. Also, the co-toxicity of some essential oils, PBO, and malathion in combination with certain conventional insecticides, bio-rational insecticides, and certain non-chemical compounds against *C. maculatus* adults were also evaluated.

Materials and Methods

1. Insect

The cowpea beetle, *C. maculatus* was reared according to (Suleiman *et al*., 2014)

2. Agents used

Malathion, Carbosulfan, Alpha- cypermethrin, Sulfoxaflor, Thiamethoxam, Emamectin benzoate, Spinosad, Indoxacarb, Hexaflumuron, Metaflumizone, PBO, Jojoba oil, Flaxseed oil, Lemongrass oil, *B. bassiana, M. anisopliae,* Talc powder, Kaolin and Malathion dust.

3. Bioassay tests

3.1. Filter paper bioassay test

Impregnated filter paper technique, described in FAO method 15 (Anonymous, 1974) and modified for bruchids by Tyler and Evans (1981). Whatman's No.1 filter papers (90 mm diameter) were used with different concentrations of insecticides as previously mentioned on *C. maculatus.* Using a 1-ml syringe, 0.7 ml of each concentration was applied to filter paper, then left to air-dry for distilled water to evaporate. Ten unsexed adult *C. maculatus* (0-48 h old) were released onto each treated filter paper and covered with Petri-dish. The weight of seeds (15g) was treated with each concentration and divided into 3 replicates. The mortality was recorded after 1,2,3,4,5 and 6 days post-treatment for each pesticide. A brush was used to gently push a cowpea beetle's abdomen and if there was no response, the insect was confirmed dead (Gbaye *et al*., 2016).

Mortality percentages were corrected by Abbot's formula (Abbott, 1925). The LC_{50s}, slope, toxicity index values were calculated by SPSS software program.

3.2. Seed-dip bioassay test

The seed-dip bioassay method was done according to (Hafez *et al.,* 2014) with little modification, a seed-dip bioassay was done against *C. maculatus* adults to determine the potency of the tested insecticides. Fifteen grams of cowpea seeds were dipped in each concentration of the insecticides for 10 seconds then the seeds were left to dry. Five concentrations were used for each insecticide and three replicates were done for each concentration. All treatments were diluted with distilled water. Ten unsexed adults (0-48 h old) were exposed to the treated seeds and covered with petri-dish. The control seeds were treated with water only. All experiments were done at a constant temperature of 29 ± 1 °C and a relative humidity of 65 ± 5% R.H. The mortality data were recorded as mentioned in filter paper bioassay.

3.3. Joint action of tested compounds and essential oils on *C. maculatus*

The joint action was carried out using the same toxicological tests noted aforementioned using serial concentrations and three replicates. The oils, piperonyl butoxide (PBO) and malathion were mixed as follows: The three essential oils, PBO and malathion were tested as synergists/ potent to the tested compounds using mixing seed method.

For all the tested dust compounds (*B. bassiana, M. anisopliae,* ascorbic acid, boric acid, talc powder, kaolin and malathion), the oils and PBO were mixed with distilled water+ Triton X100 (0.5 ml/1 liter), the seeds were dipped in the prepared solution for 10s. Then, the dust tested materials were sprinkled on the fifteen-gram treated seeds. The seeds were divided into 3 replicates, then put on the petri-dish and let it dry. The mixture rates were used as following: 1ml oil:50 ml of (solution); 10µl PBO:50 ml of (solution) ;0.005 g malathion:2 g of (dust compound). The dust concentration diluted by flour powder. Ten adults (0-48 h old) were added to each replicate. A similar sample (15 g of cowpea seeds) was dipped only in distilled water and left to dry and used as control (Abd ELrazik, 2016).

Results

1. Toxicity of tested compounds alone against *C. maculatus*

Data in Table 1 showed the LC_{50}, slope values, and probit lines of the three conventional insecticides (malathion, carbosulfan and alpha-cypermethrin) against *C. maculatus* adults (0 – 48 h old) using filter paper method. Based on the LC_{50} values, the toxicity of the tested compounds after 3 days post-treatment could be ascending as: carbosulfan> malathion>alpha- cypermethrin. Despite the low toxicity index values of the tested insecticides (0.99-22.9) compared to (100.00) in carbosulfan, the LC_{50} values were decreased with the increase of period of exposure. The results demonstrated that carbosulfan was the most effective agent against *C. maculatus* followed by malathion and alpha- cypermethrin. The high slope value was recorded for alpha-cypermethrin against cowpea beetle (2.02) while, the least one with malathion (1.23) after 3 days. All the tested insecticides have high slope values of more than 1, this indicated that the tested population of *C. maculatus* responded homogenously with them.

Table 1. Toxicity of three conventional insecticides (malathion, carbosulfan and alpha-cypermethrin) against the adults *C. maculatus* (0 – 48 h) using filter paper method after 1,2 and 3 days from treatment

Insecticides \ Toxicity	Time (day)	LC_{50} (ppm) (C. Ls95%)	Slope± SE	x^2	Sign.	Toxicity Index *
Malathion	1	3640.49 (1645.32-17837.42)	0.96±0.1	7.07	0.07	12.88
	2	1684.61 (663.64-8433.41)	0.69±0.08	6.34	0.09	7.58
	3	335.75 (66.34-1169.63)	1.23±0.09	22.11	0.00	22.9
Carbosulfan	1	468.93 (120.06-5682.73)	1.02±0.09	20.77	0.00	100
	2	127.76 (57.18 - 288.34)	1.66 ±0.12	13.51	0.00	100
	3	76.9 (6.32 - 971.34)	1.93 ±0.16	35.89	0.00	100
Alpha-cypermethrin	1	42961.45 (33735.36-61293.8)	1.79 ±0.23	1.61	0.66	1.09
	2	12560.29 (10875.37-14506.34)	2.15±0.19	5.18	0.16	1.02
	3	7726.07 (1695.28-21958.04)	2.02±0.16	30.01	0.00	0.99

* Toxicity Index (TI) = (LC_{50} value of the most toxic compound / LC_{50} value of the tested compound) × 100

The toxicity of four bio-rational insecticides (two neonicotinoids (thiamethoxam, sulfoxaflor), emamectin benzoate and spinosad) against *C. maculatus* adults (0 – 48 h old) using filter paper method is shown in Table 2.

Table 2. Toxicity of four bio-rational insecticides (two neonicotinoids+ emamectin benzoate and spinosad) against the adults *C. maculatus* (0 – 48 h) using filter paper method after 2,3 days from treatment

Insecticides \ Toxicity	Time (day)	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Toxicity index *
Sulfoxaflor	2	18741.61 (11181.7-67714.23)	1.54±0.18	6.37	0.09	18.54
	3	4155.33 (3526.51 - 4866.30)	1.80± 0.15	0.44	0.93	31.09
Thiamethoxam	3	3241.44 (2215.48-4414.61)	0.85±0.13	2.23	0.53	39.85
Emamectin benzoate	2	3475.48 (2443.51-5434.3)	0.74±0.13	2.96	0.39	100
	3	1291.75 (952.36-1657.67)	1.10± 0.13	4.28	0.23	100
Spinosad	2	9835.39 (7390.86-14413.8)	0.98±0.13	2.31	0.51	35.34
	3	1459.92 (993.39-2058.41)	1.17±0.09	8.31	0.14	88.48

*Toxicity index (TI) = (LC_{50} value of the most toxic compound / LC_{50} value of the tested compound) × 100

Based on the LC_{50} values, the toxicity of tested compounds after 3 days post treatment could be ascending as: emamectin benzoate>spinosad >thiamethoxam >sulfoxaflor. Although, the low toxicity index values of the tested insecticides (18.54-88.48) compared to (100.00) in Emamectin benzoate were observed, the LC_{50} values were decreased with the increase of period of exposure. The high slope values were recorded for all tested compounds (more than 1) except with thiamethoxam against cowpea beetle after 3 days.

Two bacterial derivatives; act as neurotoxic with different site of actions, emamectin benzoate and spinosad have more toxicity effects than two neonicotinoid insecticides, thiamethoxam and sulfoxaflor as contact insecticides.

Three IGRs (indoxacarb, hexaflumuron and metaflumizone) toxicity data are shown in Table.3 against *C. maculatus* adults using filter paper method. Based on the LC_{50} values, the toxicity of tested compounds was dramatically increased with increased days post-treatment from 3-6 days by 2-22 folds. The toxicity after 6 days could be ascending as: hexaflumuron > metaflumizone >indoxacarb. There were low toxicity index values of the tested insecticides (18.07-57.3) compared to (100.00) in hexaflumuron. The IGRs have flat slope lines, the beetle's population responded heterogenous to these insecticides.

Table 3. Toxicity of three IGR (indoxacarb, hexaflumuron and metaflumizone) against the adults *C. maculatus* (0 – 48 h) using filter paper method after 3,4,5 and 6 days from treatment

Toxicity / Insecticides	Time (day)	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Toxicity index *
Indoxacarb	3	21138.34 (13760.01-43999.11)	1.38±0.21	2.83	0.42	21.69
	4	17019.87 (8180.16-99669.69)	0.54±0.13	0.49	0.92	5.34
	5	3886.85 (2528.66-7248.29)	0.60±0.12	0.23	0.97	23.38
	6	901.91 (493.03-1329.66)	0.74±0.13	1.87	0.59	18.07
Hexaflumuron	3	4586.22 (2546.72-9985.35)	0.52±0.13	3.51	0.30	100
	4	3539.07 (2103.66-9730.88)	0.57±0.12	4.68	0.19	100
	5	908.8 (109.79-3748.57)	1.09±0.13	15.39	0.002	100
	6	163 (38.824-313.89)	0.59±0.13	2.82	0.42	100
Metaflumizone	3	10418.55 (7635.09-16092.28)	1.24±0.15	4.33	0.23	44.02
	4	6363.77 (2408.32-67345.28)	0.69±0.09	6.67	0.08	14.28
	5	1776.61 (552.4-13096.13)	0.52±0.08	5.52	0.14	51.15
	6	284.45 (8.45-848.91)	0.66±0.09	7.49	0.06	57.3

IGR = Insect growth regulators, *Toxicity index (TI) = (LC_{50} value of the most toxic compound / LC_{50} value of the tested compound) × 100

Seven neurotoxic insecticides tested using filter paper bioassay showed the highest contact toxicity compared to IGRs. Carbosulfan, a carbamate insecticide, showed the highest potent effect as contact poison, offering a strong alternative to malathion for stored seeds protection.

Using the dipping seeds method as residual bioassay, the LC_{50}, slope lines of the three conventional insecticides against *C. maculatus* are shown in Table 4. The toxicity of these insecticides was the same first rank in toxicity as the results of contact toxicity and with the same sequence except malathion take the first rank. The toxicity ascending as: malathion > carbosulfan >alpha-cypermethrin. With LC_{50} values of malathion, carbosulfan, alpha-cypermethrin recorded 21.57, 114.43. 975.18 ppm, respectively. The population of tested cowpea beetles responded homogenously with malathion and carbosulfan, showed steep probit lines (1.62-2.12), the opposite with alpha-cypermethrin that have flat line (0.83). The LC_{50} values of tested insecticides decreased by increasing exposure time.

The study analyzed the residual effects of four bio-rational insecticides against *C. maculatus* adults (Table 3). Thiamethoxam was the most effective, followed by emamectin benzoate, spinosad, and sulfoxaflor. The toxicity index values increased with exposure time, with sulfoxaflor showing the least effectiveness against cowpea beetle.

Our toxicity data agree with other studies. The potency of carbosulfan confirmed by Bogamuwa *et al*., 2002 on adults of *C. maculatus*. The effectiveness of cypermethrin with high LC_{50} values against *C. maculatus* applied on concrete (Karimzadeh *et al*., 2020). The toxic effect of cypermethrin on four strains of the red flour beetle indicated that the CTC-12 was more resistant to cypermethrin than the remaining strains.

Table 4. Toxicity of three conventional insecticides (malathion, carbosulfan and alpha-cypermethrin) against the adults *C. maculatus* (0 – 48 h) using dipping seeds method after 1,2,3 days from treatment

Insecticides \ Toxicity	Time (day)	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Toxicity index *
Malathion	1	156.33 (48.55-472.77)	0.68 ±0.06	7.07	0.07	100
	2	37.89 (10.44 - 230.98)	2.07± 0.19	46.49	0.00	100
	3	21.57 (12.48-31.55)	2.12± 0.21	7.01	0.07	100
Carbosulfan	1	661.09 (331.26-1419.5)	2.77±0.33	14.32	0.002	23.65
	2	205.76 (32.55-881.03)	1.92 ±0.12	79.59	0.00	18.41
	3	114.43 (13.02 - 564.84)	1.62 ± 0.1	79.73	0.00	18.85
Alpha-cypermethrin	1	18267.1 (14739.75-26119.71)	2.29± 0.37	0.02	0.88	0.86
	2	5459.16 (4470.48-6360.31)	2.41 ±0.33	1.44	0.23	0.69
	3	975.18 (174.29-7752.53)	0.83 ±0.07	61.42	0.00	2.21

*Toxicity index (TI) = (LC_{50} value of the most toxic compound / LC_{50} value of the tested compound) × 100

Karnatak and Khari (1991) reported that deltamethrin and cypermethrin were significantly superior among the synthetic pyrethroids and mortality was directly correlated with the dose concentration.

Although, the tested IGR insecticide is promising non-neurotoxic chemicals as contact effect, the low or moderate contact toxicity of IGRs insecticides against the adults of cowpea beetles were compatible with the studies on chlorfluazuron and hexaflumuron that have a moderate toxicity at tested concentrations (Gad *et al.,* 2022). The efficacy of tested IGR is not very high against cowpea beetles in the present study due to the fact that adult female oviposits inside the kernel, and immature development is not affected by contact insecticides (Arthur, 1996).

Contact toxicity can disturb the nervous system's work in pests that cause cell muscle paralysis, leading to the pests to stop eating and die. It can be said that neuro-pesticides cause faster death in *Callosobruchus maculatus* (F.) than IGRs. (Rehman *et al*., 2019) tested thiamethoxam and imidacloprid for the control of khapra beetle, *Trogoderma granarium* under laboratory conditions. Mortality of insects was recorded after 24, 48 and 72 hours. On treated wheat, thiamethoxam provided 82.61% while imidacloprid gave 78.18% mean mortality of khapra larvae at 2 ppm after 72 hours.

The high co-toxicity of tested dust formulations may be due to manufacturers providing insecticides as dust concentrates or formulations in the user country using local mineral carriers and imported insecticides, with stabilizing agents added for anti-caking purposes. (Mahdi and Khalequzzaman, 2012).

Table 5. Toxicity of four bio-rational insecticides (two neonicotinoids+ emamectin benzoate and spinosad) against the adults *C. maculatus* (0– 48 h) using dipping seeds method after 1,2,3 days from treatment

Toxicity / Insecticides	Time (day)	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign	Toxicity index *
Sulfoxaflor	1	6336.02 (3600.09-24435.29)	1.56 ±0.35	3.42	0.33	88.99
	2	1270.4 (605.87-6487.14)	1.12±0.13	8.69	0.03	45.26
	3	242.96 (37.75-980.42)	1.05±0.09	20.01	0.00	25.19
Thiamethoxam	2	3626.89 (1949.47-9857.86)	0.64±0.09	0.49	0.92	15.85
	3	61.19 (10.71-159.89)	0.86±0.08	9.17	0.03	100
Emamectin benzoate	1	5638.33 (3013.25-16077.02)	0.78±0.12	5.09	0.17	100
	2	574.99 (236.13-2002.71)	0.75± 0.08	6.48	0.09	100
	3	86.94 (0.00-645.31)	0.70±0.08	22.38	0.00	70.38
Spinosad	2	3557.28 (1752.88-11636.63)	0.53±0.08	2.14	0.54	16.16
	3	148.55 (35.11 - 412.37)	0.62±0.07	5.94	0.12	41.19

*Toxicity index (TI) = (LC_{50} value of the most toxic compound / LC_{50} value of the tested compound) × 100.

Data in Table 6. show the LC_{50}, slope values of the three IGR (indoxacarb, hexaflumuron and metaflumizone) against *C. maculatus* adults using dipping seeds method. Based on the LC_{50} values, the toxicity of tested compounds after 6 days post treatment could be ascending as: hexaflumuron > indoxacarb >metaflumizone. Although, the low toxicity index values of the tested insecticides (0.08-6.08) compared to (100.00) in hexaflumuron, the LC_{50} values were decreased with the increase of period of exposure. While the high slope value was recorded for hexaflumuron against cowpea beetle (1.60) after 2 days, the least one with metaflumizone (0.27) after 6 days. The LC_{50} values of tested materials decreased by increasing exposure time.

The study found that insecticides have varying LC_{50} values in bioassays, with neuro insecticides ranking first and IGRs second. Some insecticides are more suitable for contact, while others are effective in seed mixing. Carbosulfan and malathion are good store or seed protectants.

Table 6. Toxicity of three IGR (indoxacarb, hexaflumuron and metaflumizone) against the adults *C. maculatus* (0 – 48 h) using dipping seeds method after 1,2,3,4,5,6 days from treatment

Toxicity / Insecticides	Time (day)	LC_{50} (ppm) (C. Ls95%)	Slope± SE	x^2	Sign.	Toxicity index *
Indoxacarb	3	5353.75 (2409.56-22143.62)	0.52±0.09	0.41	0.94	5.47
	4	4436.69 (1877.86-21677.05)	0.44± 0.08	2.87	0.41	0.08
	5	257.11 (153.45 - 427.31)	0.53± 0.07	1.99	0.57	0.77
	6	18.97 (4.25-44.35)	0.41± 0.07	4.64	0.2	7.8
Hexaflumuron	2	776.39 (133.57-41852.78)	1.60±0.16	27.37	0.00	-
	3	292.77 (8.24-7302.44)	0.89± 0.08	27.27	0.00	100
	4	3.57 (0.00 - 37.57)	0.39±0.08	7.34	0.06	100
	5	1.97 (0.00-19.05)	0.56±0.09	8.87	0.03	100
	6	1.48 (0.00-12.28)	0.61±0.09	6.88	0.08	100
Metaflumizone	3	8598.01 (3337.81-52390.85)	0.49±0.09	1.39	0.71	3.4
	4	2764.91 (1308.82-10043.04)	0.46 ±0.08	0.63	0.89	0.13
	5	61.86 (16.71 -136.36)	0.34± 0.07	1.17	0.76	3.18
	6	24.34 (1.81 - 73.63)	0.27± 0.07	3.37	0.34	6.08

*Toxicity index (TI) = (LC_{50} value of the most toxic compound / LC_{50} value of the tested compound) × 100.

2. Toxicity of tested compounds combinations against *C. maculatus*

Some of the tested plant essential oils showed low to moderate toxic effects on the adults of cowpea beetle compared with the tested insecticide or non-toxic agents. Using essential oils as synergists to insecticides has been studied in many insect pests (Abd ELrazik, 2016). The study of synergistic pesticide combinations with plant derivatives is crucial. These combinations can be synergistic, antagonistic, or additive. Synergistic effects are greater than individual effects, while antagonistic effects are less than individual effects. Additive effects are equal or close to individual effects. (Verma *et al*., 1981).

We tested the co-toxicity effect of the combination/ mixtures of piperonyl butoxide (PBO), three essential oils (lemongrass oil, jojoba oil, flaxseed oil), plus malathion insecticide with certain selected compounds against cowpea beetle adults under laboratory conditions as shown in Table 7,8,9 and 10. The LC_{50} values and the co-toxicity coefficients of thiamethoxam, malathion and three essential oils mixtures at 50:1 mixing ratio (insecticide: oil) against adult stage of *C. maculatus* were calculated and presented in Table 8, 9 and 10. A synergistic activity was detected in thiamethoxam and + lemongrass oil + jojoba oil +flaxseed oil recording LC_{50} values (3.58, 42.53, 53.31 ppm) respectively and recording co-toxicity coefficient values (1709, 144, 115) respectively (Table 8,9 and 10). Malathion+ lemongrass oil mixtures showed increased toxicity (low LC_{50} value, 1.53ppm) and with co-toxicity value of 254 as shown in Table 15, whereas, for the rest of oil mixtures, lemongrass oil, jojoba oil, PBO mixtures with two dust entomopathogenic *B. bassiana* and *M. anisopliae,* desiccant kaolin dust showed synergistic effect with co-toxicity coefficient (195, 125, 126), (289, 148, 148,), (487, 65, 508) as shown in Table 12,13 and 15, respectively .

Table 7. Toxicity of three insecticides (alpha- cypermethrin, thiamethoxam and hexaflumuron) against the adults *C. maculatus* (0 – 48 h) using dipping seeds and mixing with PBO method after 3 days from treatment

Toxicity / Insecticides	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Co-toxicity Coefficient *
Alpha-cypermethrin	2640.60 (2111.06-3087.7)	1.92±0.27	1.73	0.63	37
Thiamethoxam	73.3 (53.49 - 98.84)	0.89±0.12	1.06	0.79	83
Hexaflumuron	831.38 (689.31-1094.73)	1.61±0.24	0.15	0.99	35

*Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the insecticide alone (Table 4, 5 and 6) by the LC_{50} of the insecticide + synergist (PBO) ×100.

Table 8. Toxicity of three insecticides (alpha-cypermethrin, thiamethoxam and hexaflumuron) against the adults *C. maculatus* (0 – 48 h) using dipping seeds and mixing with Jojoba oil (60%) method after 3 days from treatment

Toxicity / Insecticides	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Co-toxicity Coefficient *
Alpha-cypermethrin	1753.19 (1065.49 - 2285.74)	1.50±0.27	1.46	0.69	56
Thiamethoxam	42.53 (28.87 - 57.88)	0.88±0.12	5.26	0.15	144
Hexaflumuron	658.81 (544.25-853.34)	1.39±0.23	2.9	0.41	44

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the insecticide alone (Table 4, 5 and 6) by the LC_{50} of the insecticide + synergist (jojoba oil) ×100.

Various plant essential oils and major compounds were reported to have not only lethal effects, acting as pesticides against insects, mites and various arthropods, but also acting as repellents and antifeedants in addition to their adverse effect on some biological parameters such as growth rate, life cycles and reproduction (Rattan, 2010, Boulogne *et al*., 2012; El-Wakeil, 2013; Kedia *et al*., 2015). Essential oils can act as fumigant, their vapor action may also be very promising against pests of stored grain products because of their insecticidal properties (Rozman *et al.,* 2007; Perez *et al.,* 2010, Park *et al.,* 2016). The mortality of *C. maculatus* tested both by contact and by fumigation varied with the dose of the essential oil. High mortality rates and inhibition of F1 progeny production were recorded by contact with seeds treated with essential oil for *C. maculatus*. The vapors of the essential oil exhibit a strong toxic action against the adults of *C. maculatus*. It has also been established that essential oil generally remains more toxic, and its effect is persistent. The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Regnault-Roger and Hamraoui 1995; Ahn *et al*., 1998). It is also possible that various minor components may be involved in some types of synergism with other active components (Yu *et al.,* 2004).

Essential oils of sweet basil, *Ocimum basilicum* L., and African basil, *O. gratissimum* L., (Labiatae) were evaluated either alone or in combination with kaolin powder, as control agents for the cowpea beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). The findings, which complement those of Ke´ita (2000), represent an ongoing effort to provide rural people with effective and simple methods to protect stored cowpeas. In addition, the successful use of cotton and peanut oils as well as shea butter suggests that local African plant species have considerable potential for the protection of stored products (Dabire´, 1993).

This result is also similar to that of Ishaaya *et al*. (1983) who reported higher mortality of *T. castaneum* in combined doses of insecticide (e.g., trans- and cis-cypermethrin) and synergist (piperonylbutoxide) (Mondal, 1990).

In the treated seeds experiment, the tested oils, jojoba oil, flaxseed oil and lemongrass oil as well as malathion showed the same effect on the studied parameters which was significantly different compared to control, which in line with (El-Sayed *et al*., 2015).

Spinosad, however, was less toxic in the 24 h treatment to *C. maculatus* than deltamethrin, an insecticide commonly used in Burkina Faso to control this insect (Sanon *et al.,* 2010). Spinosad, a biopesticide in the naturalytes family of insecticides, is a promising alternative to other commercially available pesticides for the control of storage-insect pests. Spinosad has been successfully used for the protection of >100 major crops worldwide (Thompson *et al*., 2000) and against some insect pests of stored corn and rice (Anonymous 1993, Liang *et al.,* 2002a, 2002b). Spinosad was toxic to insects by ingestion or contact, and its action on the insect nervous system at the nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptor sites (Sparks *et al.,* 2001).

Spinosad seems to be less effective than deltamethrin, at least for short (24 h) exposure periods. As reported for other insecticides (Arthur, 1997 and 1998), the efficacy of Spinosad depends on the duration of insect exposure.

Contact insecticides are usually applied to empty bins and warehouses on floors, walls and ceilings instead of direct application to stored grains (Gunther and Gunther, 2013). Cypermethrin and dichlorvos have low persistence (Tomlin, 2009) and are suitable for use against stored product pests. (Athanassiou *et al*., 2015) tested two doses of alpha-cypermethrin and thiamethoxam (0.025 and 0.1 mg a.i./cm^2) against *Trogoderma granarium* Everts and *Tenebrio molitor* L. on concrete and found that alpha-cypermethrin was more effective than thiamethoxam. Comparing their results with the results of our study indicated that adults of *T. granarium* and *T. molitor* were more susceptible to cypermethrin than adults of *C. maculatus*.

Cumulative mortality percentages values recorded no mortality by using *B. bassinana* and *M. anisopliae* until the 3rd day post-treatment. Then, from the 3rd to the 5th day after treatments, mortality gradually increased to reach 100% for *B. bassinana* against *C. maculatus* (Abdu-Allah *et al.,* 2015).

Table 9. Toxicity of three insecticides (alpha- cypermethrin, thiamethoxam and hexaflumuron) against the adults *C. maculatus* (0 – 48 h) using dipping seeds and mixing with Flaxseed oil method after 3 days from treatment

Toxicity / Insecticides	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Co-toxicity Coefficient *
Alpha-cypermethrin	1830.99 (50.72-2953.75)	1.53±0.27	6.26	0.1	53
Thiamethoxam	53.31 (38.23 - 70.85)	0.94±0.12	1.94	0.59	115
Hexaflumuron	767.04 (617.41-1074.19)	1.29±0.23	3.69	0.29	38

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the insecticide alone (Table 4, 5 and 6) by the LC_{50} of the insecticide + synergist (flaxseed oil) ×100.

Table 10. Toxicity of three insecticides (alpha-cypermethrin, thiamethoxam and hexaflumuron) against the adults *C. maculatus* (0 – 48 h) using dipping seeds and mixing with Lemongrass oil method after 3 days from treatment

Toxicity / Insecticides	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Co-toxicity Coefficient *
Alpha-cypermethrin	1435.94 (1004.52-1781.18)	2.58±0.37	5.15	0.16	68
Thiamethoxam	3.58 (0.83-7.77)	0.76±0.13	4.66	0.19	1709
Hexaflumuron	680.17 (538.49-969)	1.12±0.22	0.93	0.82	43

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the insecticide alone (Table 4, 5 and 6) by the LC_{50} of the insecticide + synergist (lemongrass oil) ×100.

Table 11. Efficacy of two bio-agents (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc powder and kaolin powder) compared with malathion dust against the adults *C. maculatus* (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±0.1	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 the most toxic compound (malathion dust) / LC_{50} value of the tested compound) × 100

Table 12. The efficacy of two bio-agents (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc powder and kaolin) compared with malathion dust using mixing seeds with PBO method against the adults *C. maculatus* (0 – 48 h) after 3 days from treatment

Toxicity / Compounds	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Co-toxicity Coefficient *
B. bassiana	5219.36 (2703.62-7846.86)	0.65± 0.14	0.59	0.89	126
M. anisopliae	4581.29 (2416.59-6774.62)	0.7±0.14	1.4	0.71	148
Talc powder	18321.97 (7369.26-38489.46)	0.95±0.12	6.41	0.09	38
Kaolin	1011.55 (251.12-1989.66)	0.89±0.17	3.73	0.29	508
Malathion dust	4.94 (0.00-16.28)	0.96±0.14	10.54	0.02	79

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for compound alone (Table 11) by the LC_{50} of the compound + synergist (PBO) ×100

Table 13. The efficacy of two bio-agents (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc powder and kaolin) compared with malathion dust using mixing seeds with Jojoba oil (60%) against the adults *C. maculatus* (0 – 48 h) after 3 days from treatment

Toxicity / Compounds	LC_{50} (ppm) (C. Ls95%)	Slope ± SE	x^2	Sign.	Co-toxicity Coefficient *
B. bassiana	5245.67 (1945.75-9564.85)	0.56±0.12	0.45	0.95	125
M. anisopliae	4585.75 (196.45-14575.86)	0.30±0.09	1.54	0.65	148
Talc powder	10660.77 (1898.97- 22198.73)	0.66± 0.15	0.03	0.99	66
Kaolin	7850.65 (6149.6-9662.55)	1.32± 0.15	5.09	0.17	65
Malathion dust	10.13 (5.51-15.86)	0.63± 0.09	4.71	0.19	38

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the compound alone (Table 11) by the LC_{50} of the compound + synergist (jojoba oil) ×100.

Table 14. The efficacy of two bio-agents (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc powder and kaolin) compared with malathion dust using mixing seeds with flaxseed oil against the adults *C. maculatus* (0 – 48 h) after 3 days from treatment

Toxicity / Compounds	LC_{50} (ppm) (C. Ls95%)	Slope±SE	x^2	Sign.	Co-toxicity Coefficient *
B. bassiana	8892.78 (4715.27-15456.11)	0.51±0.13	0.44	0.93	74
M. anisopliae	9258.99 (5114.96-15962.24)	0.52±0.13	0.95	0.81	73
Talc powder	12175.2 (4009.19-21617.99)	0.86±0.16	0.18	0.98	57
Kaolin	8012.26 (6119.39-10049.42)	1.19±0.14	2.2	0.53	64
Malathion dust	16.42 (10.5-23.99)	0.74±0.08	4.92	0.18	24

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the compound alone (Table 11) by the LC_{50} of the compound + synergist (flaxseed oil) ×100.

Table 15. The efficacy of two bio-agents (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc powder and kaolin) compared with malathion dust using mixing seeds with lemongrass oil against the adults *C. maculatus* (0 – 48 h) after 3 days from treatment

Toxicity / Compounds	LC_{50} (ppm) (C. Ls95%)	Slope±SE	x^2	Sign.	Co-toxicity Coefficient *
B. bassiana	3349.86 (1425.53-5278.27)	0.67±0.14	0.5	0.92	195
M. anisopliae	2345.14 (827.91-3930.15)	0.68±0.14	0.08	0.99	289
Talc powder	11652.21 (7850.21-16532.78)	0.86±0.13	0.98	0.72	60
Kaolin	1056.21 (375.29-1822.8)	1.29±0.22	5.26	0.15	487
Malathion dust	1.53 (0.00-6.08)	0.92±0.14	11.96	0.01	254

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the compound alone (Table 11) by the LC_{50} of the compound + synergist (lemongrass oil) ×100.

Table 16. The efficacy of two bio-agents (*B. bassiana* and *M. anisopliae*) and two desiccant dusts (talc powder and kaolin) using mixing seeds with malathion dust (0.005 gram) against the adults *C. maculatus* (0 – 48 h) after 3 days from treatment

Toxicity / Compounds	LC_{50} (ppm) (C. Ls95%)	Slope±SE	x^2	Sign.	Co-toxicity Coefficient *
B. bassiana	117.04 (0.00-852.47)	0.47±0.47	10.39	0.02	5607
M. anisopliae	78.81 (0.00-596.84)	0.52±0.08	10.9	0.01	8624
Talc powder	557.12 (0.00-2886.01)	0.65±0.12	6.95	0.07	1260
Kaolin	831.15 (80.65-2155.39)	0.99±0.22	4.02	0.26	618

* Co-toxicity Coefficient. Calculated by dividing the half lethal concentration (LC_{50}) for the compound alone (Table 11) by the LC_{50} of the compound + synergist (malathion dust) × 100.

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فعالية بعض المبيدات الحشرية وخلطها مع المواد ذات التأثير التنشيطي ضد الحشرات الكاملة لخنفساء اللوبيا

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الملخص

تعد حشرة خنفساء اللوبيا واحدة من أكثر افات المواد المخزونة انتشارًا في ثمار البقول. أجريت هذه الدراسة لتقييم السمية المحتملة لبعض المواد الكيميائية ضد خنفساء اللوبيا باستخدام ورق الترشيح وغمر البذور. أظهرت النتائج أن مبيد الكاربوسولفان وهو مبيد حشري من الكربامات هو الأكثر سمية يليه مبيد الإندوكساكارب والملاثيون. واختلفت قيم التركيز السام النصفي في طرق التقييم المختبرة. بشكل عام، عند طرق ورق الترشيح وغمر البذور فإن جميع فئات المبيدات الحشرية المختبرة لها نفس تصنيف السمية. بينما تحتل المبيدات الحشرية العصبية المراتب الأولى ومنظمات النمو الحشرية تحتل المراتب اللاحقة. تشير هذه النتائج إلى أن بعض المبيدات الحشرية المختبرة أكثر ملائمة بالملامسة، في حين أن البعض الآخر أكثر فعالية في الخلط مع البذور مثل الثيامثوكسام. في حين أن الكاربوسلفان والملاثيون يمكن استخدامهم في حماية او تخزين البذور. كما أن مبيد ايمامكتين بنزوات والاسبينوساد يعدان عاملين صديقين للبيئة كعامل بالملامسة وتأثيرات متبقية كعامل حماية ضد الآفات الحشرية المخزنة. كانت المبيدات الحشرية العصبية هي الأعلى سمية بين المبيدات الحشرية التي تم اختبارها، في حين كانت منظمات النمو الحشرية هي الأقل سمية. بعض المبيدات الحشرية أكثر ملائمة بالملامسة، والبعض الآخر أكثر فعالية في الخلط مع البذور. يعتبر إيمامكتين بنزوات والاسبينوساد من المواد الصديقة للبيئة من حيث الملامسة والتأثيرات المتبقية. تم اكتشاف التأثير التنشيطي في الثيامثوكسام وزيت حشيشة الليمون وزيت الجوجوبا وزيت بذور الكتان.