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NANO-COPPER, TEMPERATURE, BIOPESTICIDES AND TRADITIONAL COMPOUNDS MITIGATE THE ADVERSE EFFECTS OF COWPEA BEETLE IN CORRELATION WITH GERMINATION PERCENTAGE OF COWPEA

MAHA I. EL-SAWY [1], ATTIAH Y. KERATUM[2], RAAFAT B. ABOU ARAB[1], MOHAMED F. SHADY[2] AND SHERIF M. ABDEL-DAYEM[2*]

[1]*Plant Protection Research Institute, Sakha Agric. Res. Station, ARC, Giza, Egypt*
[2]*Department of Pesticides Chemistry and toxicologs, Faculty of Agriculture, Kafrelsheikh University33516, Kafr elsheikh, Egypt*
[*]*Corresponding author's email: sherif19821@yahoo.com*

Abstract

This study aimed to evaluate the efficacy of three plant extracts (*Ficus*, camphor, and clove) and their powders compared with a pyrethroid insecticide (cypermethrin) and copper nanoparticles against the cowpea beetle *Callosobruchus maculatus*. This study also determined the effects of different temperatures on the progeny arising from immature stages of *C. maculatus* (eggs, larvae, and pupae). Toxicity, progeny, % F_1 reduction, % seed weight loss, and effect on immature stages were studied using the mixing with feeding medium method. Results showed that cypermethrin and copper nanoparticles achieved the highest mortality followed by plant powders and their extracts. In addition, these materials had the same effects on % adult reduction and % weight loss. Most of the immature stages were deterred under the studied temperatures, except for 30°C, which was the most suitable for development of the tested stages. Temperatures of 40°C and 45°C completely prevented any emergence at all durations of exposure, whereas 20°C, 25°C, and 35°C reduced the number of adult emergence. Using plant extracts and their powders provided cowpea seeds the best protection against *C. maculatus* infestation. In addition, the formulations of plant (extract/powder) and the age of deposit affected plant efficiency. Among the tested materials, clove extract had the highest effect, whereas its powder exerted the least effect. The powders were better than the extracts. Furthermore, the combination of the different tools used in the present investigation might be an effective approach of integrated pest management strategy.

Key words: Plant products, *C. maculatus*, Biology copper nanoparticles, Cypermethrin, Low and high temperatures.

Introduction

Stored product insects cause high losses in the quantity and quality of products, including grains. Their occurrence rate is approximately 5% – 10% at most regions and increases at hot regions (Haque *et al.*, 2000). Such insects cause 10%–15% inhibition of germination (Adugna, 2006). Up to 100% loss of crops is due to infestation of throb beetles, *Callosobruchus chinensis* (L.) and *Callosobruchus maculatus*, which are the major pests of cowpeas in hot countries (Raja *et al.*, 2007). The cowpea beetle *C. maculatus* (F.) belongs to Bruchidae (Coleoptera: Chrysomelidae). Beetles of this subfamily are eaters of beans (Fabiaceae). Cowpea seed beetle is the main harmful insect for stored seeds worldwide because its mature and immature stages can cause 75% grains weight loss in severe infestation and 100% damage without control (Bagheri Zonouz, 1996). Infection of cowpea beetle occurs after laying its eggs into stored beans, and then larvae burrow inside beans the day after. It feeds on all seed parts and then remains dormant there. The adult does not make any harm, whereas the larvae inflict great damage (Tran & Credland, 1995). Insects must be prevented from attacking stored seeds because they can destroy large quantities, especially with the passage of a long time. Nowadays, many safe strategies can be employed to control insect pests; one of such is the use of plant parts with good effective insecticidal activity. Natural products are used as alternatives to synthetic pesticides to minimize harm to the ecosystem (Koul *et al.*, 2008). Plant extracts have very high efficacy in breaking down insecticide resistance (Arnason *et al.*, 1989). Reducing the use of traditional pesticides is an important goal of post-harvest pest management, especially in stored food, and using non-chemical materials is recommended (Mason & Strait, 1998). Nanotechnology has attracted attention in the development of new biocidal agents. Metal oxide nanoparticles are important in the removal of risky chemical materials from the environment (Gunalana *et al.*, 2012). Temperature is a main ecological factor influencing the development and activity of bugs (Mahroof *et al.*, 2003 and Roesli *et al.*, 2003). However, the cost of mentioning a high degrees more than 39°C under 15°C are effective but it costs high of management. Under hot conditions, the metabolic rate is high; under cold conditions, pest growth is low, and productivity is decreased (Flinn & Hagstrum, 1990). Heat less than 15°C results in the death of insect pests, especially those at immature phases (Ghosh & Durbey, 2003).

Consequently, the present study aims to develop alternative control methods using nano-copper, plant extracts, and their powders compared with cypermethrin against *C. maculatus* and evaluate the efficiency of different temperatures.

Materials and Methods

Cowpea beetle (*C. maculatus*, Coleoptera, Bruchuidae): Cowpea (*Vigna unguiculata* var. Kafr El-Sheikh) were collected from Egyptian stores, sieved, and then cleaned of inner impurities. The day after, the samples were placed in a small container and in the oven at 70°C for 1 h. Then, they were spread into more containers (500 mL) each with 300–500 adults of *C. maculatus* (0–48 h old) for laying eggs and enclosed with gauze and tightened with plastic tape to prevent the insects from escaping. The containers were stored at 28 ± 2°C and 70 ± 5 relative humidity. After 7 days, the first adults were discarded, and new ones (48 h old) were used for subsequent experiments (Zayed, 2015).

Tested materials

Plant products: Extracts of ficus (*Ficus nitida* L.) and camphor (*Eucalyptus* sp. L.) leaves and clove (*Eugenia aromatic* L.) flower buds.

The leaves of *F. nitida* and *Eucalyptus* sp. and the flower buds of *E. aromatic* were cleaned with distilled water, air dried for a week, and then crushed by a local mixer. The crushed leaves were individually exposed to 95% ethanol to extract some active compounds with Soxhlet for 15–18 h. Raw active materials were filtered through a Whatman filter paper and concentrated on a rotatory evaporator under low pressure. Water was removed from the extracts by using sodium sulfate, and then the products were stored in small vials at 4°C in a refrigerator (Andy & Edema, 2019).

Plant powders: Plant powders of ficus (*Ficus nitida* L.) and camphor (*Eucalyptus* sp. L.) leaves and clove (*Eugenia aromatic* L.) flower buds. The leaves of the tested plants were cleaned and several parts equal in size and put in outside lap for air dried and day fifth transferred to the oven at 60°C for the same period (Sowunmi, 1983). The dried plant parts were crushed individually using a blender and passed through a 40 hole/mm^2 mesh to obtain powder. Large pieces were crushed again and sieved.

Insecticides used: Cypermethrin (Cyper.) (20%): Common Name: Cypermethrin, and Trade name: Cypermethrin.

Chemical N: α-cyano-3-phenoxybenzyl, 2,2 dimethyl-3 (3,2 dichlorovinyl) cyclopropane carboxylate. Produced by: Changzhou Good-Job Biochemical Co. Ltd., China.

Nanoparticles (Copper nanoparticles): Purchased from Nano Tech. laboratory.

Physical method: Temperature experiment: This experiment was designed to assay six ranges of temperature at three times of the exposure effect of the biology of immature stages of *C. maculatus*. Cowpea seeds (20 g) were placed in jars (100 mL) and then infested with 10 mature, unsexed *C. maculatus* (24–48 h age). All parents removed after 10 days. The glass jars were divided into three groups. The first group was exposed directly to different temperatures (20°C, 25°C, 30°C, 35°C, 40°C, and 45°C) at three periods (10, 15, and 20 min). The second group was exposed to the same temperature and time after 10 days of infestation. The third group was exposed to the same conditions after 15 days of infestation. After treatment, all jars were left under laboratory conditions until the emergence of adult insects. Each treatment and control was conducted in three replicates.

Methods of application: Seed treatment (mixing with feeding medium): This test was performed to evaluate the efficiency of the plant extracts and powders, copper nanoparticles, and cypermethrin against *C. maculatus* on feeding medium. For seed treatment, the concentrations dissolved in acetone were 5.0, 10.0, 15.0, and 20 ppm for cypermethrin; 25000, 50000, 100000, and 150000 ppm for plant extracts; 0.5%, 1.0%, 2.0%, and 4.0% w/w for plant powders; and 0.1%, 0.2%, 0.4%, and 0.8% w/w for cupper nanoparticles. Each concentration of the tested material was mixed with 20 g of cowpea seeds placed in jars (250 mL). The jars were shaken by hand to ensure mixing the grains with the tested materials. Jars without any tested materials (solvent only) served as control. All treatments and control were conducted in triplicate. Each jar was added with 20 new adults of *C. maculatus* (0–48 h old), enclosed with muslin, and then stored in laboratory conditions as mentioned above. Mortality counts were recorded after 2, 4, and 8 days for plant extracts; 1 and 3 days for plant powders; and 1, 2, and 3 days for cypermethrin and copper nanoparticles. Mortality was calculated using Abott's formula (Abbott, 1925):

$$\% \text{ Correct mortality} = \frac{\% \text{ Mortality of treatment} - \% \text{ Mortality of control}}{100 - \% \text{ Mortality of control}} \times 100$$

LC_{50} and LC_{90} with confidence limits and slope values were calculated for all tested materials.

Effect on the progeny (preventive way): In this experiment, 20 g of cowpea seeds of each treatment were cleaned and tempered to a moisture content of 12.5% ± 0.5%. The toxicants tested at LC_{90} concentration were mixed with cowpea seeds. Then, 20 unsexed adult insects of *C. maculatus* (0–48 h old) were added to each treatment. After 10 days, all adult insects were removed for each treatment. Then, all jars were stored under laboratory conditions.

The average number of eggs found on cowpea seeds, eggs hatched, and emerged adults and the reduction % in adult numbers of *C. maculatus* were recorded and calculated using the equation of El-Lakwah *et al.*, (1992).

$$\% \text{ Reduction} = \frac{\text{Mean no. of emergent in control-mean no. of emerged adults in treatment}}{\text{Mean no. of emerged adults in control}} \times 100$$

Effect on weight loss: The % Loss of cowpea after the emergence of F_1 progeny was calculated as described by Harris and Lindblad (1978).

$$\% \text{ Loss} = \frac{\text{Cowpea seeds weight before treatment - Cowpea seeds weight after treatment}}{\text{Cowpea seeds weight before treatment}} \times 100$$

Germination percentage: From each damaged and undamaged grains samples (100) were taken and placed in Petri dishes, put in a germination cabinet to determine the germination (%).

Statistical analysis

Data were analyzed statistically using SPSS (2016), and the means were compared using Duncan's multiple range test.

Results and Discussion

Effect of plant extracts on *C. maculatus*: In this study, we compared the LC_{50} values of plant extracts and cypermethrin. As shown in (Table 1), cypermethrin exerted the best insecticidal effect on *C. maculatus* with an LC_{50} of 7.43 ppm, followed by the camphor (61279 ppm) and clove (67573 ppm) extracts at 2 days after treatment. After 4 and 8 days, the clove extract had the highest effect with an LC_{50} value of 48987 ppm, followed by camphor with 58600 ppm. At 4 days after treatment, the ficus extract had no effect on *C. maculatus.* After 8 days of treatment, clove remained to have the highest LC_{50} value of 30660.9 ppm, followed by camphor with 34185 ppm and ficus at 130590 ppm. These results are in line with the results of previous studies that insecticides exert stronger effects than plant extracts. The pesticide cypermethrin at high and low doses can cause high adult mortality in *C. chinensis*. Neem oil, *Aloe vera*, and chilli can deter *C. chinensis* without nontoxic effects (Tahir & Anwar, 2015). The LC_{50} values of cypermethrin and dichlorphos on *C. maculatus* were 1286.96 and 1350.55 mg/L, respectively (Karimzadeh *et al*., 2020).

Effect of plant powder on *C. maculatus*: As shown in (Table 2), camphor achieved the highest effect on *C. maculatus*, followed by ficus and clove, with LC_{50} values of 2.27%, 3.93%, and 13.4%, respectively, after 24 h of treatment. Ficus had the highest effect after 3 days of treatment with an LC_{50} value of 1.24%, followed by camphor at 1.33% and clove at 1.58%. The results indicate that the formulation of plant (extract/powder) and the age of deposit affect plant potency. For example, clove extract exerted the highest effect, whereas clove powder exhibited the lowest effect. Moreover, the powder formulation was better than the extract one (Tables 1 and 2). Many plant parts and oils can be used as bioinsecticides against stored product pests (Hill & Schoonhoven, 1981, Desmarchelier, 1994). Extracts coated on seeds may interfere with the natural growth of pests by inhibiting their vital processes and the hormonal system. Previous studies reported that plant parts can inhibit the growth of stored insects (Hall & Harmann, 1991) by blocking the breathing openings of insects, leading to suffocation. Moreover, *Lippia nodiflora* and *Piper longum* cereals treated 14 days after hatching can cause the highest adult mortality rates on *Sitophylus oryzae* of 53.8% and 53.5%, respectively (Nalini *et al*., 2010). Hexane and methanol extracts of *Eucalyptus camaldulensis* at 5 g/kg of seeds cause a 100% death rate of insects at 6 days after addition to the grains (Mahama *et al*., 2018). In addition, aqueous extracts of *Eugenia caryophyllus*, *Bryophyllum pinnatum*, *E. camaldulensis*, and *Xylopia aethiopica* induce insect mortality of 71.21%, 81.42%, 80.00%, and 100.00%, respectively, at 5 mL doses of aqueous plants (Omotoso, 2008). Ethanol extracts of *Allium sativum* L. (Garlic), *Cordia millenii* Baker (Manjack), *Monodora myristica* (Gaertn.) (Nutmeg), *X. aethiopica* (Dunal) (Negropepper), and *Zingiber officinale* Roscoe (Ginger) show lethal effects on *C. maculatus* (Edwin and Jacob, 2017). Ethanolic extracts of *Euphorbia balsamifera* and *Lawsonia inermis* cause 96.67% and 90.00% adult mortalities on *C. maculatus* at 14 days post-treatment. Clove is widely used as a spice, but it also shows high effect on insect control without toxic effects on humans (Naveena *et al*., 2006). *Citrus sinensis* exerts the maximum weevil death on *C. maculatus* after 24 h of treatment, but *Azadirachta indica* and *Cymbopogon citratus* are the least toxic (Ojebode *et al*., 2016). Extracts of *Acorus calamus* var. *angustatus* and *Thymus mandschuricus* (Lamiaceae) against *C. chinensis* can cause 100% death at 24 h after treatment (Kim *et al*., 2003). Rather than dosage, exposure duration is the factor with the greatest influence on the poisonous properties of *Acorus* oil fumes. Various plant-released compounds have become more effective in pest resistance when compared with pesticides (Arnason *et al*., 1989, Ahn *et al*., 1997). Extracts from *A. calamus* var. *angustatus*, *Acoras gramineus*, *Cinnamomum cassia*, and *Cinnamomum sieboldii* exert good insecticidal action on adults of *S. oryzae* and *C. chinensis* (Kim *et al*., 2003). Hexane extract of cashew kernels can cause 100% death of *C. maculatus* in cowpea seeds at 96 h after treatment (Dimetry & El-behery, 2018) and Adedire *et al*., 2011).

Table 1. Insecticidal effects of camphor, ficus and clove extracts on *C. maculatus* adults after 2, 4 and 8 days of treatment.

Material	LC 50 (µg/cm^3)	Confidence limit		Slope value
		Lower	Upper	
	2 day			
Camphor	61279	47076	78180	1.284
Ficus	-	-	-	-
Clove	67573	57683	79178	1.95
	4 days			
Camphor	58600	45269	73727	1.284
Ficus	-	-	-	-
Clove	48987	41120	56906	2.05
	8 days			
Camphor	34185	13545	35391	1.05
Ficus	130590	104472	163237.5	2.96
Clove	30660.9	41120	56906	2.05

Table 2. Insecticidal effects of camphor, ficus and clove powd on *C. maculatus* adults after 1 and 3 days of treatment.

Material	LC 50 (%w/w)	Confidence limit		Slope value
		Lower	Upper	
	1 day			
Camphor	2.27	1.87	2.92	2.08207
Ficus	3.93	2.96	6.1	1.39
Clove	13.4	10.89	16.48	1.42
	3 days			
Camphor	1.33	1.18	1.50	2.7
Ficus	1.24	0.99	1.55	2.2
Clove	1.58	1.29	1.97	1.46

Table 3. Insecticidal effects of cypermethrin and copper nanoparticles on *C. maculatus* adults after 1, 2 and 3 days of treatment.

Material	LC 50 (µg/cm³)	Confidence limit Lower	Confidence limit Upper	Slope value
Cypermethrin	**1 day**			
	12.96	10.62	16.58	1.46
	2 day			
	7.43	5.86	8.92	1.9
	3 day			
	2.84	1.65	3.815	2.619
Cupper-nano particles	**1 days**			
	2040	140	2430	1.189
	2 day			
	970	550	13550	1.216
	3 day			
	550	260	840	1.36

Effect of cypermethrin on *C. maculatus*: As shown in (Table 3), the mortality percentage of cypermethrin increased with increasing concentration and prolonged contact. At the highest concentration (20 ppm), the mortality percentages were 66%, 88%, and 99% at 1, 2, and 3 days after treatment. Cypermethrin exerted the greatest prevention effect on weight loss. Previous studies were in the same trend of the present study at all the doses tested. The hexane extract of *E. camaldulensis* completely inhibited the progeny F1 production of *C. maculatus*. Most plant extracts reduced significantly the rate of seed damage and seed mass losses compared with the negative control (Mahama *et al.*, 2018).

Effect of copper nanoparticles on *C. maculatus*: As shown in Table (3), the impact of copper nanoparticles increased with high dosage and exposure time where the LC_{50} reduced from 2040 (1 day) to 550 (3 days) post-treatment. In addition, this study showed that the copper nanoparticles had the highest effect at 3 days post-treatment with an LC_{50} value of 0.055, followed by ficus (1.24% w/w), camphor (1.33% w/w), and clove powder (1.58% w/w). In addition, fumed silica airsol 200 nanoparticles at 1 g/kg caused 100% death in *C. maculatus* adults at 48 h after the treatment (Doaa *et al.*, 2015). Unlike our findings, death rates are 30% and 82% when treated with 25 µg/mL zinc oxide nanoparticles and foliage extract (Brook, David, and Vinothini Balasubramanian, 2017).

As shown in Tables 1, 2, and 3, cypermethrin exerted the strongest insecticidal activity on *C. maculatus* among all the tested materials. *Chromolaena odorata* root powder has high recorded effective mortality on cowpea weevils after 3 days exposure (Osa & Geogina, 2016). Another study showed that 5 g/100 g each of *Carica papaya* leaf powder and *Nona muricata* seed powder show the most activity in managing cowpea seed damage from *C. maculatus* (Lawal Ibrahim *et al.*, 2018). The best plant powder causing the mortality of *Oryzeaphilus surinamensis* L. (78.89%) is *Conyza discorides* (Omran *et al.*, 2020). Malgorzata & Anna (2015) mentioned that the plant parts of *Mentha pipertia* L., *Artemisia abinthium* L., *Salvia officinalis* L., and *A. sativum* L. are the most effective at high concentrations, causing the maximum death in the saw-toothed seed beetle. In the current study, results showed a significant difference in the number of eggs laid by *C. maculatus* on treated and control cowpea seeds. Among the different treatment concentrations, cypermethrin induced the highest effect on egg number, followed by clove and camphor extracts. Ficus exerted the least effect on the loss of cowpea weight (2.666 ± 0.17) compared with clove (0.667 ± 0.17) and camphor (1.166 ± 0.17). Another laboratory study observed the maximum percentage of oviposition deterrence in *Jatropha curcas* leaf extract (64.16%), *Brassica juncea* seed extract (64.65%), *A. indica* seed extract (65.44%), custard apple leaf extract (65.95%), and custard apple seed extract (67.19%) at 5% concentration (Chudasama *et al.*, 2015). The hexane fraction of *Callistemon rigidus* caused the total inhibition of F_1 progeny emergence. No progeny emergence was observed at all doses (Danga *et al.*, 2015).

LC_{90} residual effect of plant extracts and cypermethrin on the biology of *C. maculatus*: (Table 4) presents the effect of cowpea seed treatment with LC_{90} for clove, camphor, ficus, and cypermethrin on the number of eggs laid, eggs hatched, reduction percentage, and weight loss. Cypermethrin induced the highest effect on egg number (1 ± 0.58), followed by clove, camphor, and ficus compared with the control. Similarly, the hatched rate of eggs laid by exposed females significantly decreased than unexposed females. The number of hatched eggs was lower on clove treated cowpea seeds (25 ± 1.53) than camphor (32.33 ± 0.67) and ficus (186.33 ± 5.81), whereas the amount of hatched eggs was zero on cypermethrin treatment. The offspring reduction percentages of *C. maculatus* adults were 100%, 86.12%, 80.21%, and 8.19% for cypermethrin, clove, camphor, and ficus, respectively. Cypermethrin prevented the weight loss of cowpea seeds compared with the control.

LC_{90} residual effect of plant powders and copper nanoparticles on the biology of *C. maculatus*: (Table 5) presents the effect of cowpea seed treatment with LC_{90} for clove, camphor, ficus, and copper nanoparticles on the number of eggs laid, eggs hatched, reduction percentage, and weight loss. Copper nanoparticles induced the highest effect on egg number (9 ± 1.15), followed by ficus (27 ± 3.21) and camphor (30.33 ± 1.45), in comparison with the control (334 ± 2.09) eggs/20 adults. The hatched rate of eggs laid by exposed females also significantly decreased than unexposed females. The number of hatched eggs was lower on the cowpea seeds treated with ficus (12.667 ± 7.86) than on those treated with camphor (20.667 ± 3.47) and clove (39.667 ± 0.74), whereas the number of hatched eggs was 1.667 ± 9.32 on the cowpea seeds treated with copper nanoparticles. The offspring reduction percentages of *C. maculatus* adults were 99.88%, 96.8%, 94.33%, and 90.69% under the treatments of copper nanoparticles, ficus, camphor and clove, respectively. Copper nanoparticles totally prevented any weight loss followed by ficus, camphor, and clove. Data obtained in this study showed that copper nanoparticles induced the highest deterrent effect on egg number, followed by ficus, camphor, and clove powders. Clove exerted the lowest effect on the loss of cowpea weight (3.95% ± 0.08%) compared with the other tested powders. Treatment with copper nanoparticles did not show any weight loss. These consequences agree with the findings of Wazid *et al.*, (2020), who revealed that silica green nanoparticles at 1500 ppm concentration record the least average number of eggs per 100 seeds (0.71 eggs/100 seeds) and the highest in untreated check (173.22 eggs/100 seeds). At the same concentration, they reported that silica green nanoparticles had the least average seed weight loss (0.05%) compared with the untreated seeds (48.10%). Rahman *et al.*, (2020) found that the fertility of *C. maculatus* is reduced in pests mixed with nickel-oxide nanoparticles in a dosage-dependent method. For example, the highest fecundity is shown in insects bred on seeds of control, whereas the lowest fertility is found in insects bred on seeds treated at 40 ppm (NiO NPs). Many studies have recorded the important plant powders used to protect stored grains. The *C. papaya* leaf powder and *C. sinensis* powder are active on adult appearance and adult death of *C. maculatus* at 2 months of storage (Lawal Ibrahim *et al.*, 2018). Plant powders and crushed leaves of *Bulinus senegalensis*, *Cleome viscosa*, and *Hyptis spicigera* exhibit inhibitory and ovicidal effects on the egg laying of cowpea weevil females (Doumma, 2012). The inhibitory and ovicidal active on the egg laying of cowpea weevil females is dependent on the dose of phytochemicals used. This hypothesis is important with freshly ground leaves of *B. senegalensis* and *C. viscosa* (Doumma, 2012). Cowpea seeds mixed with the least dose of ground *Moringa* leaves can significantly decrease the number of eggs laid by adult insects. This condition can be clarified by using the least dose of ground *Moringa* leaves, preventing the females from laying eggs (Malaikozhundan & Vinodhini, 2017).

Table 4. The effect of LC_{90} for plant extracts and cypermethrin admixed with cowpea seeds on biology of *C. maculatus* before insect infestation.

Treatment	LC 90 (µg/cm³)	No. of eggs laid/20	No.of egg hatched	No. of emerged adults	% Reduction	% Loss of cowpea
Control	0.0	334 ± 2.08^{a}	298.33 ± 1.45^{a}	129.66 ± 1.45^{a}	0	5.0 ± 0.00^{a}
Clove	205507	40.0 ± 1.53^{e}	25.0 ± 1.53^{d}	18.0 ± 0.58^{e}	86.12 ± 0.41^{b}	0.667 ± 0.17^{e}
Camphor	582859	79.0 ± 1.15^{d}	32.33 ± 0.67^{d}	25.66 ± 0.88^{c}	80.21 ± 0.68^{c}	1.166 ± 0.17^{d}
Ficus	353162.5	227.33 ± 3.18^{b}	186.33 ± 5.81^{b}	119 ± 1.15^{d}	8.19 ± 1.73^{e}	2.666 ± 0.17^{b}
Cyper.	2.6	1 ± 0.58^{f}	0	0	100 ± 0.00^{a}	0

Table 5. The effect of LC_{90} for plant powders and copper nanoparticles mixed with cowpea seeds on biology of *C. maculatus* before insect infestation.

Treat.	LC_{90} (%w/w)	No. of eggs laid/20	No. of egg hatched	No. of emerged adults	% Reduction	% Loss of cowpea
Control	0.0	334 ± 2.09^{a}	286.33 ± 5.64^{a}	300.67 ± 0.52^{a}	0.0 ± 0.00^{e}	5.0 ± 0.00^{a}
Clove	11.94	52.33 ± 3.18^{c}	39.667 ± 0.74^{a}	28.0 ± 3.29^{b}	90.69 ± 0.97^{ab}	3.95 ± 0.08^{b}
Camphor	4.0	30.33 ± 1.45^{d}	20.667 ± 3.47^{a}	17.667 ± 4.65^{b}	94.33 ± 4.77^{d}	1.33 ± 0.16^{c}
Ficus	4.68	27.0 ± 3.21^{d}	12.667 ± 7.86^{b}	9.667 ± 7.45^{c}	96.8 ± 7.34^{c}	0.55 ± 0.016^{e}
Cu. Nano	0.4	9.0 ± 1.15^{e}	1.667 ± 9.32^{d}	0.33 ± 4.76^{d}	99.88 ± 0.11^{a}	0.0 ± 0.00^{f}

Table 6. Effect of temperatures on emergence ariseform egg stages of *C. maculatus* at the indicated periods.

Period	Temperature						Mean
	20°C	25°C	30°C	35°C	40°C	45°C	
10 min.	220.00 ^{d}	242.00 ^{c}	266.00 a	94.67 ^{g}	0.0 ^{j}	0.0 ^{j}	137.11 ^{a}
15 min.	147.33 ^{f}	195.33 ^{e}	246.33 b	85.67 ^{h}	0.0 ^{j}	0.0 ^{j}	112.44 ^{b}
20 min.	84.67 ^{h}	147.33 ^{f}	219.33 d	77.67 ^{i}	0.0 ^{j}	0.0 ^{j}	88.17 ^{c}
Mean	150.67 ^{c}	194.89 ^{b}	243.89 ^{a}	86.00 ^{d}	0.00 ^{c}	0.00 ^{c}	

LSD = 0.05; P = 1.054; T = 1.491; P × T=2.583

Effect of temperature on emergence of immature stages of *C. maculatus* at the indicated periods: Physical methods play an important role in protecting stored products during storage. These methods include temperature, moisture, and others. The present study investigated the effect of different temperatures against adults of *C. maculatus* (eggs, larvae, and pupae). A laboratory experiment including three treatments was carried out to evaluate the impact of temperatures on the F_1 progeny emerged from the immature stages of *C. maculatus* (eggs, larvae, and pupae). All the temperature degrees used showed high efficiency on the number of emerged adults. Meanwhile, exposure period was the major factor that influenced the produced progeny of F_1 with all treatments (Ahmady *et al.*, 2016).

Egg stage: As shown in (Table 6), 40°C and 45°C completely prevented any progeny of the treated eggs of *C. maculatus* at all times. Similarly, 30°C was the optimum degree that produced the highest number of progeny (243.89), whereas 20°C, 25°C, and 35°C significantly reduced the F_1 progeny compared with 30°C, at which the mean numbers of adults emerged were 150.67, 194.89, and 86.00, respectively. Ahmad *et al.*, (2019) found a decrease in mass obtained in the grains treated with garlic and ginger, followed by those treated with lemongrass powder, whereas the check treatment exerted the maximum damage. Laboratory assessment of powders from *Datura alba*, *Calotropis procera*, and *C. odorata* was performed at 2.5% and 5.0% against *R. dominica* on rice seeds at 28°C. Most treatments significantly decreased the quantity of mature insects emerging from the seeds. Data obtained in the present study clearly showed that temperature suppresses the infestation of *C. maculatus.* The effect of temperatures in the current study included three categories. The first was high temperatures (40°C and 45°C), which completely prevented any emergence at all periods of exposure (10, 15, and 20 min). The second category (20°C, 25°C, and 30°C) significantly reduced the number of F_1 progeny. The third category involved one level of temperature (30°C), which was the best suitable condition for all stages to develop and produce the maximum number of progeny. In addition, the least response stage was the eggs. Many studies investigated the efficacy of physical factors, such as temperature, and their results were in line with our findings.

Larval stage: The highest temperatures (40°C and 45°C) had the highest deterrent effect, followed by 35°C, 20°C, and 25°C at the all periods (Table 7). In hot weather, the metabolism rate increases; in cold weather, the development of insects is slow, and fertility decreases (Flinn and Hagstrum, 1990). To obtain 100% percentages of *C. maculatus* at 50°C, 55°C, 60°C, 65°C, 70°C, and 75°C after exposure to insects for 40, 25, 25, 20, 15, and 10 min, respectively (Ahmady *et al*., 2016). Raising the temperature to 50°C led to the death of 100% *C. maculatus* adults within 12 min. This difference may be due to the difference in the thermal sensitivity of the studied group (Herein Bhalla *et al*., 2008). The effect of exposing 100 infested bean seeds with different immature stages of *C. chinensis* (either eggs, larvae, or pupae) for different durations (15, 30, 45, 60, 75, 90, 105, and 120 min) to a high temperature of 50°C was investigated. The emerged progeny of adults in these infested samples and the progeny reduction percentages after treatment were determined. All the tested immature stages were affected by that high temperature level of 50°C treatment, and the most affected stages were larvae and pupae, whereas eggs were more tolerant to high temperature (50°C).

Pupal stage: As shown in (Table 8), the effect of temperature at the pupal stage increased with increasing exposure periods, except for 30°C, which achieved the highest level of emerged adults. The mean numbers of emerged adults after the treatment of different immature stages decreased with as the exposure time was prolonged (Tayeb *et al.*, 2018). All stages of *C. maculatus* are sensitive to an exposure period of 6 h at 50°C (Herein Bhalla *et al.*, 2008). Exposure of *Sitophilus* genus at 50°C negatively affects their survival and offspring production and the possibility of its successful use as a physical measure in controlling storage insects (Marijana *et al*., 2011). Temperature greatly influenced *S. oryzae* and its life span as an insect of stored grain products. A high effect is exhibited at high temperatures in comparison with low levels (Mansoor *et al.*, 2017). In general, the egg stage had the least response to the tested temperatures at all the exposure periods, followed by the larval and pupal stages.

Germination percentage: (Fig. 1) shows the germination of cowpea seeds with different treatments. The germination of all treatments was high count ranging from 67 to 97%. However, the germination for control treatment decreased to 14.6% germination. The damaged grains showed reduced germination and the sprouting of these seeds was very weak and could not stay alive for a few days after germination i.e. they die immediately in the germination cabinet. The germination for ficus powder, nano copper and cypermethrin treated grains showed the highest germination percentage.

Conclusion

This study used three plant extracts and their powders, chemical insecticide cypermethrin, and copper nanoparticles under low and high temperatures to check their efficacy in controlling insects in cowpea seeds. All the tested factors achieved promising results for the conservation of seeds. Cypermethrin and copper nanoparticles achieved the highest mortality. Plant extracts have registered less effect than their powders. Temperature showed a detrimental effect with all the tested immature stages. This study suggests using a combination of these treatments to offer a safe and cheap way to protect cowpea seeds.

Table 7. Effect of different degrees of temperature on immature stage of *C. maculatus* was exposure for 10 min.

Period	Temperature						Mean
	20°C	25°C	30°C	35°C	40°C	45°C	
10 min.	144.33 g	231.67 d	264.00 a	91.67 h	0.0 m	0.0 m	121.94 a
15 min.	84.67 i	200.67 e	243.33 b	64.00 k	0.0 m	0.0 m	98.78 b
20 min.	73.33 j	167.33 f	236.33 c	45.00 i	0.0 m	0.0 m	87.00 c
Mean	100.78 c	199.89 b	247.89 a	66.89 d	0.0 e	0.0 e	

LSD = 0.05; P = 1.172; T = 1.657; P × T= 2.870

Table 8. Effect of different degrees of temperature on immature stage of *C. maculatus* was exposure for 15 min.

Period	Temperature						Mean
	20°C	25°C	30°C	35°C	40°C	45°C	
10 min.	105.00 d	106.00 d	213.00 a	95.00 e	0.0 j	0.0 j	86.50 a
15 min.	85.00 f	92.67 e	194.33 b	82.00 f	0.0 j	0.0 j	75.67 b
20 min.	70.00 h	77.33 g	186.00 c	54.33 i	0.0 j	0.0 j	64.61 c
Mean	86.67 c	92.00 b	197.78 a	77.11 d	0.00 e	0.00 e	

LSD = 0.05; P = 1.298; T = 1.835; P × T= 3.179

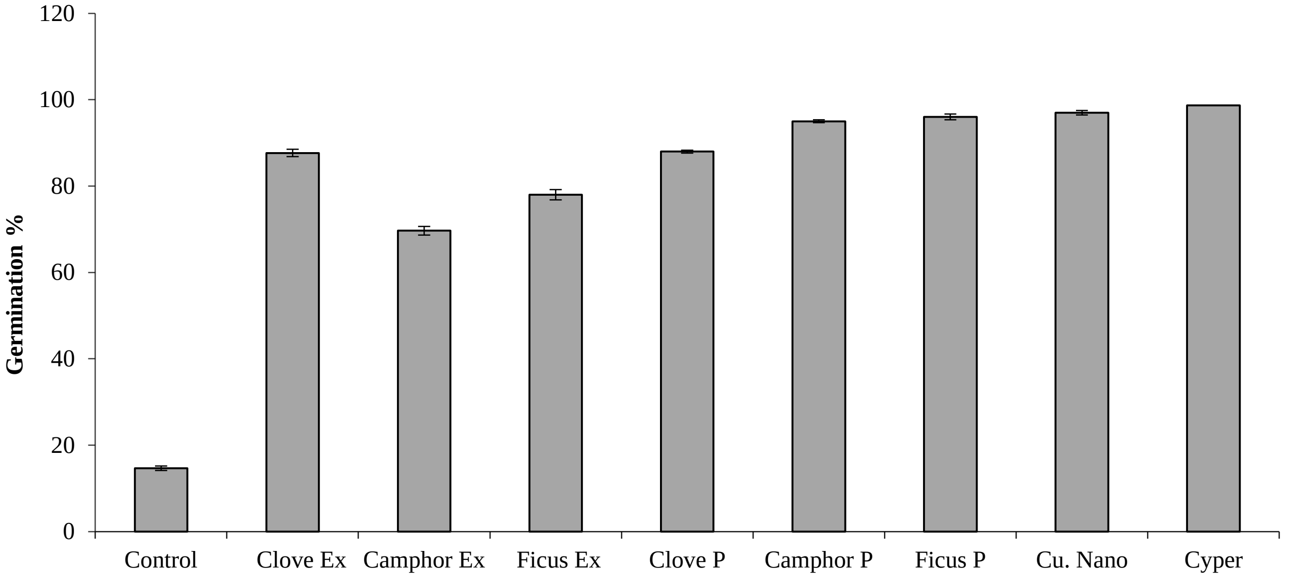

Fig. 1. The effect of plant extracts and their powders, nano copper and cypermethrin admixed with cowpea seeds on seeds germination.

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