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Impact of *Allium sativum* on *Callosobruchus maculatus* Adult Emergence, Egg Laying and Protectant Potency of Stored Cowpea

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Abstract

Pulses are considered a great protein source in Asia and the majority of other developing countries around the world. Post-harvest damages and quality impairment due to storage pests pose a significant challenge for agricultural communities in Middle East countries. As a replacement for the constructed pesticide and bulb oil extract of garlic, *Allium sativum* (L.) was examined contra the cowpea pest *Callosobruchus maculatus*. To determine the mortality percentages of cowpea beetles, as well as the oviposition, adult appearance, level of seed rotten, and Weevil Perforation Index (WPI), we mixed garlic powder at proportions of 1, 3, and 5/20 g of black-eyed peas, and used the acetone extract of garlic bulbs at 1, 2, and 3 ml for each 20 g of cowpea seeds. Three days into the treatment, 60.7%, 73.3% and 100% of the adult death in *Callosobruchus maculatus* was triggered by 1, 3, and 5/20 g of black-eyed peas, respectively. After three days of treatment, black-eyed peas died at every concentration of garlic bulb extracts. In both the powder and bulb extracts of garlic, complete preservation of seeds and prevention of adult apparition were noted. The current study proved that both powder and garlic bulb extract successfully inhibited the effect on cowpea beetles, C. maculatus, in stored black-eyed peas.

Keywords: *Allium sativum*, Bioactivity, *Callosobruchus maculatus*, Mortality, WPI.

Introduction

Callosobruchus maculatus is a serious economic pest of stored legumes, the infestation starts in the field with seeds, and the protein and carbohydrate content of the grains is reduced due to the infestation caused by the larvae of *Callosobruchus maculatus*, which leads to a decrease in their nutritional value and forces farmers to sell their crop at a low profit after harvest (1). Statistics indicate that losses caused by *Callosobruchus maculatus* in stored legumes are 35%, 7-13% and 73% in Central America, South America and Kenya, respectively (2). Legume seeds stored for five months or more can be exposed to 70% seed infestation and about 30% severe crop losses, making them unfit for human consumption. However, no detailed and complete real information is available about the crop status and economic injury level (EIL) of *Callosobruchus maculatus* under storage conditions (3). *Callosobruchus maculatus* can also be used in the rearing of bio control agents such as *Catolaccus hunteri* of pepper weevil (*Anthonomus eugenii*) (4). The biologically active components of garlic, which have spread throughout the world, include volatile organic sulphur compounds, polysaccharides, flavonoids, and pectin. Of these, allicin is the most powerful compound, and it works well against a variety of pests (5).

Garlic has spread all across the world. People should not grow garlic using chemical fertilizers because of its insecticidal properties. This is because higher fertilizer doses lower the concentration of garlic's useful ingredients. It possesses properties that are nematicidal, insecticidal, fungicidal, anti-feedant, bacterial, and repellent Pulses are considered a nutritious protein source in Asia and the majority of other developing countries around the world (6). As an alternative to commercial synthetic pesticides being used to safeguard seeds from post-harvest corruption caused by different pests and rodents, applying natural methods of seed preservation is gaining significant importance due to their low cost and availability at susceptible times.

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Natural methods of seed preservation also have fewer side effects and seem to lack the health risks that threaten humans and domestic animals (7). Fumigants are considered the main economical method for controlling pests in storage areas, but most insect pests are rapidly developing become resistant to the applied pesticides (8). Botanical plants have insecticidal properties, but progress in isolating toxic ingredients and enhancing their ability to synthesis insecticides remains disappointing. Compared to synthetic products, materials extracted from plants are more cost-effective and easier to use as insecticides due to their readily biodegradability. However, using natural materials for grain preservation requires a high concentration, often exceeding consumer taste thresholds (9-11).Insect pests like beetles, moths, and weevils can damage grain storage, reducing grain weight and compromising seed quality. Bruchids, particularly Callosobruchus beetles, can invade cowpea seeds, affecting food security. Insect remains, dead beetles, larval exoskeletons, and pupae cocoons can also contaminate human food grains, containing carcinogenic components like methoxy quinines, methyl, and ethyl, which cannot be altered by boiling (12-14). Different studies observe that Asia produces the garlic, *Allium sativum* (L.), and then transports it to other countries of the world, including Europe (15, 16). Under laboratory conditions, increasing the concentration of *A. sativum* L. leaf extract effectively increases *Tylenchulus semip* mortality. *A. sativum* (L.) also significantly hinders the movement, feeding, and reproduction of nematode populations (17), while *A. sativum* oil provides excellent protection for plants against ground nematodes. One can consider *A. sativum* a magnificent low-cost, non-poisonous pesticide for the garden, possessing naturalistic properties such as pesticides and fungicides, which can effectively control pests. Fertilizers minimize the potential for the vital components of garlic to control pests. For instance, using *A. sativum* (17, 18) can effectively manage insects such as beetles, borers, termites, ants, whiteflies, aphids, caterpillars, armyworms, and slug's .The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae), plays a crucial role in protecting black-eyed peas in various countries across the world because of the favorable climate (19, 20). *Callosobruchus maculatus*, a beetle that infects cowpeas is a significant pest in various countries. It can cause weight loss in stored cowpeas without preventive measures. Researchers have explored the Using essential oils for seed conservation to prevent pest resistance and ensure safe grain storage. The intricate action mechanism of essential oils prevents pests from developing resistance and promotes seed conservation (21-23).Consequently, there is an increasing demand to re-assess the bioactivity of *Allium sativum* (L.) oil and powder in order to prevent cowpeas from pest invasions. Current research studies are designed to investigate the efficiency of *A. sativum* (L.) bulb extract powder and oils and the determination of optimal dose level of *A. sativum* (L.) bulb extract oils and powder that can supply suitable safety for cowpea against beetle invasion.

Methodology

Insect Cultures

Adult cowpea weevil *C. maculatus* (F.) (Coleoptera: Bruchidae) adults were gathered from various stores in Babylon, Hillah City, Iraq and raised on the black-eyed peas, *Vigna unguiculata* (L.) seeds. A suitable quantity of the black-eyed peas was obtained from a public market and were kept in a freezer for a period of time up to 4 days at -16 °C in order to prohibit any other insect pests before applying the bioassay. Cowpea seeds were kept in a freezer for a specific period in order to prevent the every phase of the life cycle stages, especially the eggs, as they are highly sentient to low temperatures (24). The sterilized cowpea grains were then left to dry in the insectary to inhibit decomposition (12). Before starting all the laboratory work, about 750 g of the disinfested seeds were kept in 10 individual Kilner jars that were previously cleaned. 100 adult beetles of *C. maculatus* were inserted into each Kilner jar and coated by the muslin grasped in site by the lids of the jars, which guaranteed good aeration in the jars and stopped the beetles from flying out of the jars. After that, the jars were stored at 30±2C° and 75±2% relative humidity in a lab. After 3 weeks, the jars were evacuated from all the dead and active cowpea grain beetles, and the jars were supplied with the seeds again. At the end of the first month, adults of *C. maculatus* started to emerge, and these new adults were utilised for various experiments in current studies.

Plant Collection

The current in vitro studies used *Allium sativum* (L), also known as garlic. We procured fresh garlic bulbs from the stores in Hilla City, Babil Provence, Iraq. Bulbs were dehydrated in the researches unit and converted into a quite soft powder utilizing a special blender (Supermaster, Model SMB 2896, and Germany). The crushed was sifted and passed through 1 mm2 punctures (25). The powder was kept in containers made of plastic and stored at 3 C° in the refrigerator prior to use.

Acetone Extracts of *A. sativum*

The extract of the garlic bulbs was utilized by applying the cold extraction process. 300 g of the plant powder was drenched individually in the extractor bottle with 72% acetone in it. The mixture was repeatedly stirred with a rod made from glass Three days were needed to complete the extraction procedure, by utilising two layers of filter papers (Whatman No. 1), filtration was performed, a rotary evaporator operating at 38 °C was used to evaporate the acetone alcohol. While the rotary speed was kept at 6 to 7 rpm for 8 hours, the extract was kept until completely dry to take off any remaining solvent (26).

The Toxicity of Garlic Powder

Weighing 1, 3 and 5 g of *A. sativum* powder were taken for each of 5, 15, and 25% w/w concentrations and incorporated into a 25 g of uninfected and undamaged cowpea seeds in 300 ml containers, while there was no garlic powder for the seeds being used as a control (27). The containers with the grain and garlic powder were shaken gently to make sure complete mixing of cowpea seeds and treatment powder. 10 pairs of adult beetles were inserted into every container. To distinguish between the sexes, methods utilized in earlier studies were followed (28). A complete randomized design was utilized, and there were three replicates of the treatments, with one untreated control. After every 24 hours, adult mortality was recorded for four days, and on the 5th day, both the dead and active insects were taken out of the containers. The oviposition was recorded prior to reverting the seeds into separate containers. Within 6 weeks, the adult emergence F1 was registered, and the rate of mature insect's emergence was calculated using the equation number [1]. The cowpea seeds were weighed again on day 43, and the percentage of lack in weight was calculated as well, and the equation number [2] was utilized to calculate the impaired seed percentage. The Weevil Perforation Index (WPI) of *C. maculatus* was evaluated by utilizing the methods of both (12) the weevil puncture was calculated using the equation number [3].

$$\%\ \text{Adult emergence} = \frac{\text{No. of adults emerged}}{\text{No. of eggs laid}} \times 100 \quad \ldots\ldots\ldots\ldots[1]$$

$$\%\ \text{seed damage} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times 100 \quad \ldots\ldots\ldots\ldots[2]$$

$$\text{WPI} = \frac{\%\ \text{treated cowpea seeds perforated}}{\%\ \text{control cowpea seeds perforated}} \times 100 \quad \ldots\ldots\ldots\ldots[3]$$

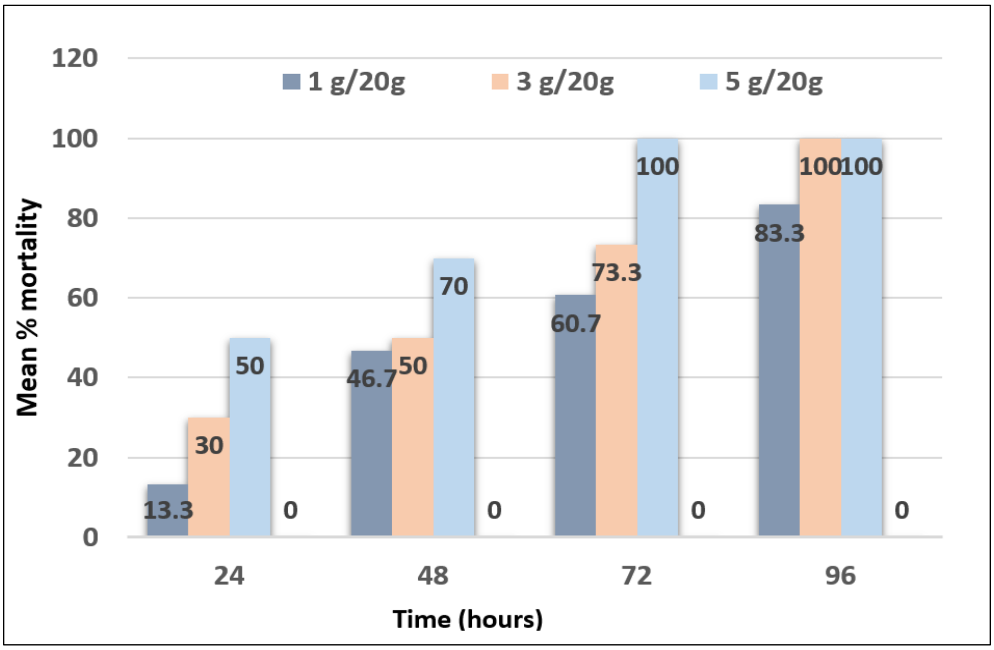

Figure 1: Impact of *A. sativum* Garlic Crushed on Adult Death-Rate of *C. maculatus,* after 24-96 hr Post Treatment

Toxicity of Garlic Bulbs Extract

A 20g of the black-eyed peas were kept in 300ml containers with garlic bulb extract in acetone with concentrations of 1, 2, and 3%. The toxicity of garlic oils against adults of *C. maculatus* was analyzed. To ensure that there were equal layers of garlic extract on the black-eyed peas, the oil was combined by a glass bar for 10-12 minutes. All the canisters were kept opened for half an hour to ensure complete removal of acetone. 10 pairs of beetles were inserted into the containers. Beetles were recorded dead for 24 hours over 4. Each treatment included 3 replicates as well as the controls, and all of them were put in a complete randomized design. The insect death index was noticed when no reaction was monitored after the palpation with the brush.

Statistical Analysis

The data was subjected to analysis of variance, and the New Duncan's Multiple Range Test was used to separate out treatment means where the results revealed significant differences (29).

Results

Effect of Garlic Powder on the Cowpea Weevil, *C. maculatus*

The efficiency of garlic powder on the death average of the beetle *C. maculatus* was noted and recorded (Figure 1). Various garlic powder concentrations were observed which showed that more than 60% mortality in comparison with control groups which had 0 % mortality after three days of treatment. According to the observations *A. sativum* powder showed about 60.7 %, 73.3 % and 100 % mortality of cowpea pest at levels of 1 /20 g, 3 /20 g and 5/20 g of cowpea grains respectively after three days of treatment. The killing rate of cowpea beetles reached 100% when using garlic powder at a concentration of 3/20 g and 5/20 g of black-eyed bean

The Influence of Garlic Powder on Egg Laying and Adult Appearance of the Cowpea Weevil, *C. maculatus*

After the impact of *A. sativum* powder on the black eyed pea significant reduction in the number of eggs laid by the *C. maculatus* was observed as compared to the control set which reached about 90.1% of adult emergence (Figure 2). The cowpea seed which were addressed with *A. sativum* powder Showed 0% of adult emergence except for the one which was treated with 1 g of *A. sativum* that showed 15.2% of adult emergences.

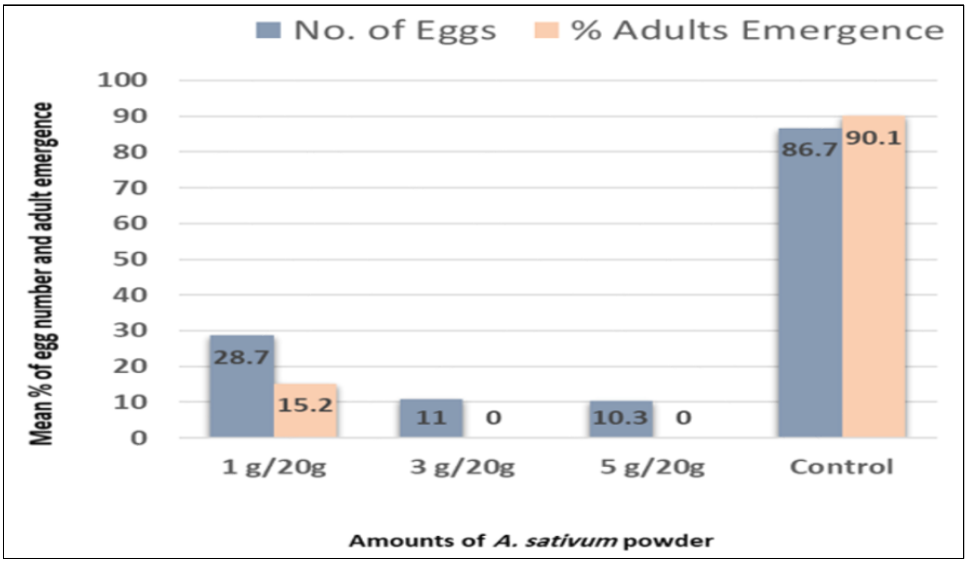

Figure 2: Effect of Garlic Powder on Egg Laying and Emergence of the Cowpea Weevil *Callosobruchus maculatus*

The Influence of Garlic Extract on *Callosobruchus maculatus*

According to the observations, the garlic extract at various concentrations caused 100 % death rate of adult *C. maculatus* insects except for the concentration of 1 ml for each 20 g of the black-eyed peas resulted in 80% adult mortality after three days of treatment and 100% of adult mortality was observed after four days (Figure 3).

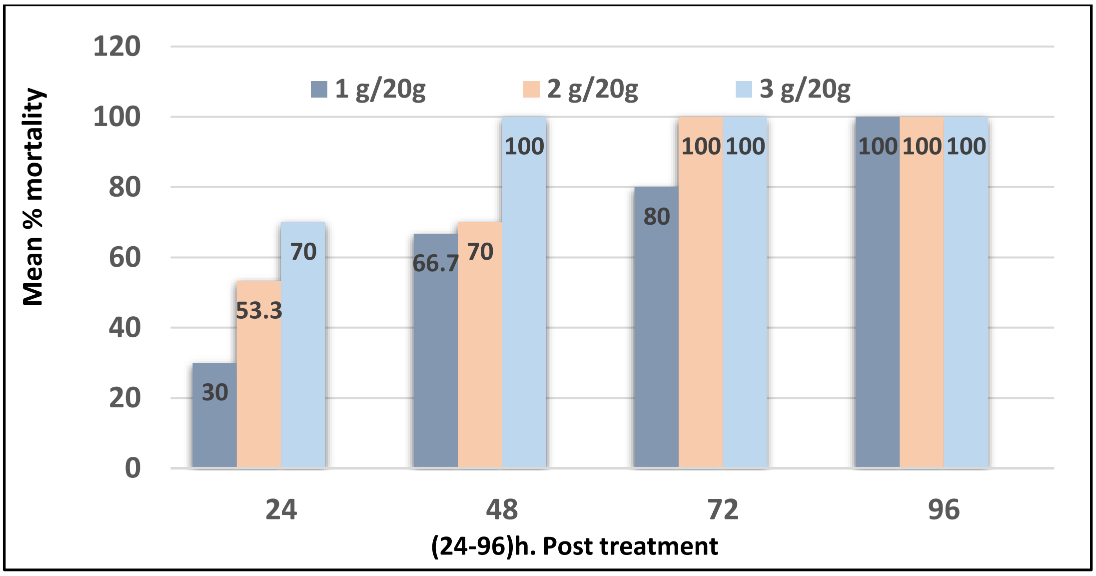

Figure 3: Impact of Garlic *A. sativum* Extract on Death Rate of the Adults of *C. maculatus*

Effect of Garlic Bulb Extract on Egg Laying and Adult Development of *C. maculatus*

According to the observations, the number of eggs laid by *C. maculatus* adults were reduced significantly when treated with garlic extract as compared with the control group of cowpea seeds where almost 85 number of eggs were found. From other side, remarkably high percentage of adult emergence was observed in the control group, the percentage recorded was ($P > 0.05$) from each of the treatments by recording 0.0 adult emergence (Figure 4). It was clearly observed that the garlic extract completely deterred the growth of (F1) beetle.

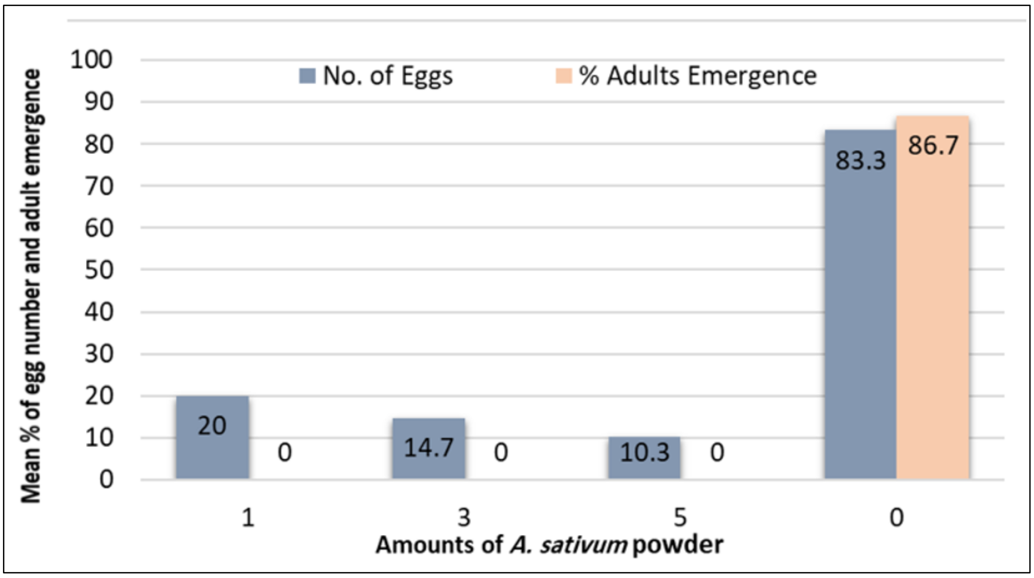

Figure 4: Impact of Garlic Extract on Egg Laying and Emergence of the Mature Cowpea Weevil Adults

The Deterioration Estimation of Cowpea Seeds Addressed with Garlic Powder, and Extract

Regarding the management of damage caused by *C. maculatus* (Table 1) that indicate no significant variation (P > 0.05) was observed in the black-eyed peas treated with garlic powder Whereas the (WPI) of 3 figured on cowpea seeds to which garlic powder was added at a rate of 1g was significantly different (P>0.05) from the WPI of the control group of the black-eyed peas.

Table 1: Protectant Potency of the *Allium sativum* Powder on Cowpea Seed

Garlic crushed	g/20g cowpea	The rate of the total number of grains	Rate of Corrupted grains	% of grain corrupted	% loss of weight	WPI.
Allium sativum	1	93.5	4.0+0.0[a]	4.2+0.4[a]	0.7+0.3[a]	5.2+0.1[a]
	3.0	92.5	0.0+0.0[a]	0.6+0.0[a]	0.8+0.0[a]	0.0+0.0[a]
	5.0	95.6	0.0+0.0[a]	0.0+0.0[a]	0.0+0.0[a]	0.0+0.0[a]
Control	0.0	95.4	78.6+0.3[b]	83.2+0.2[b]	85.9+0.2[b]	50.0+0.0[b]

Note: There is non- significant impact at (p>0.05) for mean in the same column that are followed by the same letter. *When the value of weevil perforation index (WPI) is less than 50 it means the effect is with a positive indicator, while if the WPI value more than 50 it means a negative indicator.

Table 2: Protectant Potency of the Garlic *Allium sativum* Extract on Cowpea Seed

Garlic crushed	g/20g cowpea	The rate of the total number of grains	Rate of Corrupted grains	% of grain corrupted	% loss of weight	WPI.
Allium sativum	1	95.1	0.0+0.0[a]	0.0+0.0[a]	0.7+0.3[a]	0.0+0.0[a]
	3.0	94.7	0.0+0.0[a]	0.6+0.0[a]	0.0+0.0[a]	0.0+0.0[a]
	5.0	92.8	0.0+0.0[a]	0.0+0.0[a]	0.0+0.0[a]	0.0 +0.0[a]
Control	0.0	94.3	69.9+0.1[b]	75.4+0.4[b]	84.5+0.3[b]	50.0+ 0.0[b]

Note: Ther are non- significant impact at (p>0.05) for mean in the same column that are followed by the same letter. *when the value of weevil perforation index (WPI)is less than 50 it mean the effect is with a positive indicator , while if the WPI value more than 50 it mean a negative indicator.

In (Table 2), no Corrupted to legumes or loss of weight was observed when seeds were treated with concentrations of that were applied in current studies. The WPI was 0.0 for the examined garlic extract compared to control group of seeds.

Discussion

Plant-based pesticides are effective against insect because they contain Essential oils and component that can either contact the insect's body surface or penetrate its cuticle out of the thin areas of its body, that reason palsy and finally death, or they can spread and penetrate between the insect's body tissues in a similar method to that of pesticides (30).

It is also Botanical insecticides include of harmful, alkaline/effective components, which prevent insects from feeding and ultimately lead to their death. Additionally, entering through the respiratory system has an impact on the neurological and digestive systems (31). According to the results of the research, it was observed that *A. sativum* powder and oil can be considered a highly toxic material to the black-eyed peas beetles due to its intense choky smell that can damage the respiratory system of insects and it may lead to suffocation and death later (32). Furthermore, a rise in adult mortality had been noticed and its cause has been linked to elevated extract concentrations (33). Also, significant differences were found between garlic extract and powder during the treatment, which was expected due to the differences in the chemical composition of the plant extract under study and the physiological composition of the insects. Moreover, the effectiveness of the plant extract was affected by the timing of application Numerous scientific researchers have provided explanations for variations in action, which aligns with the findings of the present research, the duration of exposure may help clarify this, or it may be related to the extent of penetration of these components or active compounds into the body, the quantity of active ingredients it contains may be the cause of this toxicity (34). Found that garlic contains terpenoids that act as insecticides, causing insect death due to loss of appetite resulting from a severe reduction in the respiratory activities of insects (35), another chemical substance in garlic is allinin inhibits the activity of cholinesterase in pests. Found comparable outcomes regarding the effectiveness of *A. sativum* oil against Trogoderma granarium and hose flies (36). *A. sativum* was proven to be an effective pesticide against *Epilachna verivestis* (37). According to previously reported studies it is found that 86% of adult mortality of *Sitophilus zeamais* and other adults of maize weevil occurred after the treatment of methanol extract of *A. sativum* (37, 38).

two reason behind reduce rate of oviposition with absence of adult emergence in the addressed seed by garlic bulbs oil extract ,the first one reason movement might be inhibited due to the presence of oil which affected the mating process (11 , 25). Second causes, that the ingredients in the extracts modify or change the ovaries, thereby preventing oviposition (36). Volatiles can infiltrate the egg through its breathing pores potentially reducing the hatching efficiency (39), also, non-volatile compounds obstruct these pores in a manner akin to that of volatile substances, suffocating the embryo, Due to presence of the extracted oil, eggs lost the capacity to adhere to the surface of the cowpea seeds, as well as it prohibited the offspring output. However, the decrease in F1 adults in seeds mixed with garlic powder at rate 1g can be the result utilized concentration. In general, the ability to work as a larvicidal as well as ovicidal of both the garlic crush and oil can be returned to the existence of lipophilic combinations (40). The physical properties of oil can clog the pores of the respiratory system, the spiracles, which lead to high eggs mortality. Whereas the physical and chemical toxicity of garlic bulbs oil can be the main reason of its larvicidal activity (41).

Conclusion

A. sativum powder and oil can provide entire protection for the cowpea seeds against *C. maculatus*. The capacity of garlic powder or oil, in the seed's protection, was very noticeable. In addition to the ovicidal, the larvicidal ability of the powder or oil of the plant being tested in the current study was behind the entire killed for the small amount of eggs which were placed, and percentage of few others that could hatch to larvae from transfer to the pupal phase.

Abbreviations

Nil.

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Author Contributions

Nada Abd Karbel: Methodology, Samerh Shaker Hamood: Methodology, Wurood Alwan Kadhim: Software and formal analysis, Al Shareefi E: Writing—original draft preparation, Nebras Mohammed Sahi: Writing—review and editing, Al Shareefi E: Supervision, Nebras M Sahi: Supervision.

Conflict of Interest

All authors declare no conflict of interest in current study.

Ethics Approval

Not applicable

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