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EFFICACY OF THREE BOTANICAL POWDERS AS MUNG BEAN GRAIN PROTECTANTS AGAINST *CALLOSOBRUCHUS MACULATUS* (FABRICIUS) (COLEOPTERA:BRUCHIDAE)

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Abstract

Laboratory experiment was carried out in Marshes Researches Center lab in University of Thi–Qar in Iraq during May 2019, to evaluate for three botanical powder effects, Cinnamon (*Cinnamomum Cassia*), Cubeb (*Piper Cubeba*) and Mint (*Mentha* spp.) against southern cowpea beetle *Callosobruchus maculates* on Mung bean stored seeds. The experiment carried out according to factorial precision in complete randomized design (CRD) to estimating the Mortality ratio and the complete randomized design (CRD) to estimating seed weight losses ratio and seed germination ratio. The results showed that there was significant effect for all treatment powders of *Callosobruchus maculatus* mortality ratio on the Mung Bean seeds except the mass fractions of Mint powders was not significant, the results showed that the highest average mortality ratios of insect was 52.58 and 62.47% to the mass fraction (4g powder) for Cinnamon and Cubeb powder respectively, For the exposure periods, the results showed that the highest mortality ratio of the insect was 100% for the periods 9-12, 8-12 and 10-12 days for Cinnamon, Cubeb and Mint powders respectively. The overlapping effect of exposure durations and mass fractions, the highest ratio reached 100% for all the mass fractions for the periods 9-12 days of Cinnamon powder and it reached 100% for mass fraction of 4grm powder for the periods 7-12 days and mass fractions 0.5, 1and 2g powder for the periods 8-12 days for Cubeb powder and it reached 100% for mass fractions 0.5, 1and 2g for the periods 9-12days and mass fraction of 4g for the periods 10-12 days. The effect of infestation with insect of Seed weight loss was significantly, the highest average of percentage loss in the weight of seeds and reached 5.62% after a period 35 of days of storage. The results showed that there were no significant effects in averages of germination ratio of Mung Bean seeds treated with vegetable powders under consideration.

Key words : *Callosobruchus maculatus*, Cinnamon, Cubeb, Mint, mortality ratios, loss weight, germination ratio of Mung Bean seeds.

Introduction

The Southern Cowpea beetle *Callosobruchus maculatus* (Fabricius) is the most important pests of the seeds stock crops, also the field's crop in some areas (Hill, 1990). Gujar (1976) finds the seeds infestation of 20 varieties of legumes, affect the seeds germination by the larvae which damages the seed contents and wastes secrete that decay substances reaches the proportion of the larval consuming (29-45.6%) of the total weight seeds. The insect lays (76-107 eggs) at the temperature of 30C° with moisture 70% the eggs hatch during (4-6 days) to larvae that develops inside the seeds and feeds on them until they appear clearly near the surface, makes a hole by their rodent jaws gets out of the hole a complete insect to re-cycle life again (Doria and Ravos, 1975). The insect is characterized by its multiple generations per year and the infestation severity of the seeds reaches 11 generations per year (Lale and Vidal, 2001). One pair of insects cause the seeds weight loss of 61.4% during four weeks, besides the infestation seeds are not suitable for human nutrition (El-Degwi and El-orabi, 1997; Poeke, 2004). The chemicals pesticides are the most frequently used to control pest in granary, due to the destruction of the ozone layer by these pesticides, it has led to the thought of alternative safe methods and materials, such as using of plant materials that using powders, water extracts or organic solvent extracts (Alhag, 2000). Belko (1994) is mention That the use of powders is easy It is useful to

that protect the seeds of cowpea when stored as they are counted from modern methods and cover the seeds in the form of layers, which helps in reducing the incidence of Southern Cowpea beetle *Callosobruchus maculatus* (F.). El-Lakwah *et al.,* (1992) use black pepper powder with a concentration of 4% added to grains to prevent the infestation of stock insects, also Allotey (2004) finds that using of The Sweet Orange peel powder *Citrus sinensis* (Linn.) Obseck gives protection for Cowpae seeds against the southern Cowpae beetle *C. maculatus* for more than three months, also Kayode (2006) explains when used the hot and the cold water extracts of *Giliricidia sepium* against Southern Cowpea Beetle *C. maculatus*, the hot water extract had the greatest effect against the insect than the cold water extract. The using of 0.5% (weight/weight) of the crude acetone extract of *Striga harmontheca* (Del) Benth against *c. maculatus* lead to a reduction in the eggs number placed on Cowpae seeds to 48% and reduction in the appearance of adults to half (Kiendrebeogo *et al.,* 2006). Alhag (2000) confirms that the plant extracts of the leaves of the Harmel, Neem seeds, Rammaram and Clove plant had an effect to prevent the laying of eggs, the percentage of productivity and a decrease in the offspring resulting to cowpea beetle by 13.4, 12.4, 11.9, 11.5%, respectively and number of emerging adults stood at 9.33 and 11 respectively, while the control treatment number was 15 insects. It was also finds by (Mahmood, 2017) when he tested for the vital efficacy of the brocade extract *Calotropis procera* against the southern Cowpae beetle *Callosobruchus maculates* that the extract had a repellent effect against the insect, also It has a good effect in decreasing the number of eggs placed by the female insect. Due to the importance of the insect and as a result of the significant damage caused to the legumes in general and to find alternatives to chemical pesticides of plant origin, the powders of some plants were used, study of their effects on the loss of the insect and the percentage of loss in the weight of the affected seeds and the extent of the effect of powders on the germination rate of the Mung Bean seeds.

Materials and Methods

Laboratory experiment was carried out in Marshes Researches Center lab in University of Thi–Qar in Iraq during May 2019.

Insect rearing

The seeds of Mung bean infested with the southern cowpea beetle *Callosobruchus maculates*, were obtained from the local markets, and the insect was diagnosed with the classification keys, and 10 pairs (males +females) were removed and added to 250g of the seeds, which were pre-sterilized and sterile in the electric oven at 60C° and for two hours for the purpose of obtaining a pure colony of the insect, then placed in clean glass jars and covered with cloth and attached to a rubber band placed in the Incubator at temperature of 30±2C° and relative of 70 ± 5% taking into account the renewal of the colony continuously (Guntrip and Sibly, 1998). A pupas group was isolated in a glass jar covered by a cloth attached to a rubber strap and placed in the incubator under the same conditions as the production of adults of the same age to be used in the experiment afterwards.

Preparation of Plant powders: Collected parts of the plants used in this study, namely (Cinnamon, Cubeb, Mint) and as shown in the table 1 (of the local markets of Al-Nasiriyah city) pure of impurities and dried in laboratory conditions and grinding by a domestic electric mill to a very fine powder individually and put in bags in the freezer until use by method (Al-Dosari *et al.,* 2008).

Table 1: The names of the plants from which they were manufactured for the plant powders used in the study.

Local plant name	Scientific name	Family	Used plant part
Cinnamon	*Cinnamomum cassia*	Lauraceae	Bark
Cubeb	*Piper cubeba*	Piperaceae	Seed
Mint	*Mentha* spp.	Lamiaceae	Leaves

Test the effect of plant powders on mortality ratios of the southern Cowpea beetle *Callosobruchus maculatus*

The plant powders were weighted with a delicate balance in the Mass fractions (0.5, 1, 2, 4) grams of plant powder and each powder separately, 50g of the sterile Mung Bean seeds were placed in glass jars and added a vegetarian powder with three replicate per treatment and then shake well to mix the powder with the seeds, the comparison treatment was without adding any powder to it, and each package was supplied with 5 pairs (males + females) of the insect randomly selected and all of which were taken from a farm where the pupas were raised, the glass jars were covered with a piece of cloth fitted with rubber strap, and according to the method (Appel *et al.,* 1999). The results of the death of the insects were recorded every 24 hours for 12 days and for the period from 9/5/2019 to 20/5/2019, the rates of death were adjusted according to the Abbotte equation (Abbotte, 1925).

Estimation of the percentage loss of the weight of the Mung Bean seeds of the southern Cowpea beetle *Callosobruchus maculates*

A amount of clean and injury-free Mung Bean seeds were taken and treated at 60C° for two hours (50g for the sample and four replication), placed in glass jars and added 5 pairs (males + females) of the insect randomly selected and all of them at one age taken from a farm where It raised the pupas (except sample comparison, which was left without adding any insect on it), was covered with a cloth and attached to a rubber band and preserved under the conditions of breeding itself and the weight loss was calculated weekly for 35 days according to the following equation:-

Loss of seed weight % =

$$\frac{\text{Primary weight} - \text{Final weight}}{\text{Primary weight}} \times 100\%$$

(Keita *et al.*, 2001)

Effect of plant powders on the percentage of Mung Bean seeds germination for highest Mass fraction only

Several random seeds were selected from the powder-treated Mung Bean seeds (4g powder/50g seeds), for this purpose sterile and clean petri dishes were placed with distilled water filtered paper and the seeds were put on it and allowed to germinate (10 seeds per dish and four replications), the treatment of the comparison included healthy and sterile seeds without powder with four replications, the percentage of the germination was calculated a week later (Brasil, 1992).

Statistical analysis

The data was analyzed statistically using precision factorial experiments in complete randomized design (CRD) in Experiment No.2-3 (after adjusting the proportions of mortality ratios by Abbotte equation) (Abbotte, 1925), Experiments No.2-4 & 2-5 were statistically analyzed using complete randomized design (CRD), compared averages by the least significant difference (L.S.D) 0.05 by (program genestat statistical analysis).

Results and Discussion

Effect of tested plant powders on mortality ratios of the southern Cowpea beetle *Callosobruchus maculatus*

Effect of tested of Cinnamon powder on mortality ratios of the southern Cowpea beetle *Callosobruchus maculatus*

The results of the analysis of variance table 2 showed that there was a significant effect of mass fractions of Cinnamon powder, exposure duration and overlap between mass fractions and exposure duration in average of mortality ratio insect among the Mung Bean seeds. The results showed that the effect of Cinnamon powder was higher in giving the highest average mortality ratio of *Callosobruchus maculatus* insect among the Mung Bean seeds as it amounted 52.58% to the mass fraction (4g powder) while the lowest average mortality ratio of insect it was 39.69% to the mass fractions (1g powder). Jumbo, *et al.,* (2018) showed that oil dosage of Clove and Cinnamon increments proportionately decreased the growth rate and reduced the losses in bean weight caused by cowpea weevils, and offspring emergence was almost abolished when parents were exposed to the LD_{20} of each essential oil and It was found that mortality was reached 100% when the insects were exposed to doses of LD_{50} for clove (67.6 μL kg^{-1} of bean) and cinnamon (139.4 μL kg^{-1} of bean) essential oils. Al-Saadi (2004) found that Increasing the weight of botanical powders caused an increase in ratio of mortality of *Callosobruchus maculatus* insect. As for the exposure periods, the results showed that the highest mortality ratio of the insect was reached 100% for the period 9-12 days after the insect was exposed to the powder, while the lowest mortality ratio of insect was 0.83% for the period one day after the insect was exposed to the powder. noteworthy that the highest of insect mortality ratio was after 8 days of exposure to the powder up to 100% after 12 days, exposure to the insect powder. Ismail, *et al.,* (2014) mentioned According to their obtained results, it could be stated that the tested compounds played an important role in controlling the bruchid *C. maculatus*. Bhaduri, *et al.,* (1985) found that some botanical extracts were significantly efficient in decrease the numbers of *Callosobruchus maculatus*. Al-Mansur *et al.,* (2009) found that the mortality ratio of the white Termite insect increases with increasing exposure to plant extracts also the highest mortality ratio of clove extract in the period was 4 days. The overlapping effect of exposure durations and mass fractions of Cinnamon powder on the average of insect mortality ratios, the highest of mortality ratio reached 100% and for all mass fractions of Cinnamon powder and for period 9-12 days of the exposure, while the lowest of insect mortality ratio was 0% during 1 and 6 days for all mass fractions of Cinnamon powder except the mass fraction 4g that had the highest of insect mortality ratios compared the other mass fractions. Mahdi and Rahman (2008) indicated that admixture of 3% of turmeric and cinnamon powder (w/w) with black gram seeds, caused 100% mortality and decreased F1 progeny of *C. maculatus* (F.)

Effect of tested of Cubeb powder on mortality

Table 2: *Callosobruchus maculatus* mortality ratios using different quantities of Cinnamon powder on Mung Bean seeds (corrected according to Abbote equation).

Exposure duration (days)	Mass fractions of Cinnamon powder (gram)				Average of exposure duration	$LSD_{0.05}$
	0.5	1	2	4		
1	0	0	0	3.33	0.83	14**
2	3.33	3.33	10	10.33	6.75	
3	7.33	18.33	29.33	33	22	
4	16.33	23.67	32	44	29	
5	14	14.33	34	29.33	22.92	
6	0	0	0	16.67	4.17	
7	16.67	0	33.33	27.67	19.42	
8	50	16.67	66.67	66.67	50	
9	100	100	100	100	100	
10	100	100	100	100	100	
11	100	100	100	100	100	
12	100	100	100	100	100	
Average of Mass fractions	42.31	39.69	50.44	52.58	$LSD_{0.05}$ Mass fractions * Exposure duration	
$LSD_{0.05}$	8.08**				28**	

Table 3: *Callosobruchus maculatus* mortality ratios using different quantities of Cubeb powder on Mung bean seeds (corrected according to Abbote equation).

Exposure duration (days)	Mass fractions of Cinnamon powder (gram)				Average of exposure duration	$LSD_{0.05}$
	0.5	1	2	4		
1	0	0	3.33	6.67	2.5	8.58**
2	6.67	10	13.67	20.33	12.67	
3	18.33	25.67	22	29.33	23.83	
4	27.67	28	23.67	35.33	28.67	
5	25	10.33	10.33	19	16.17	
6	0	0	0	39	9.75	
7	89	72.33	83.33	100	86.17	
8	100	100	100	100	100	
9	100	100	100	100	100	
10	100	100	100	100	100	
11	100	100	100	100	100	
12	100	100	100	100	100	
Average of Mass fractions	55.56	53.86	54.69	62.47	$LSD_{0.05}$ Mass fractions * Exposure duration	
$LSD_{0.05}$	4.95**				17.15**	

ratios of the southern Cowpea b.eetle *Callosobruchus maculatus*

The results of the statistical analysis table 3 showed a significant effect on the mass fractions of Cubeb powder, exposure duration and overlap between mass fractions and exposure duration in average of insect mortality ratio among the Mung Bean seeds. The results showed that the highest average mortality ratio of *Callosobruchus maculatus* insect was 62.47% to the mass fraction (4g Cubeb powder), while the lowest average mortality ratio of insect was 53.86% to the mass fraction (1g powder). The deadly effect of Cubeb powder may be due to its volatile oils, Simpson and Ogorzoly (2001) pointed out that The deadly effect of *Piper nigrum* powder may be due to its volatile oils and the most important compound where Flandrin and Dibetin that the distinctive smell of pepper is due to this oil also it contains chemical compound piperine refer hot spicy taste to it. Al-Kinany (2014) mentioned in her study that The highest corrected killing ratio of *Callosobruchus maculatus* insect was at the mass fraction 4g and the lowest corrected killing ratio was at the mass fraction 0.5g of powder of *Piper nigrum*. For the exposure periods, the highest mortality ratio of the insect was 100% for the period 8-12 days of the Cubeb powder exposure, while the lowest mortality ratio of insect was 2.5% after one day when the insect was exposed to the Cubeb powder. It is noted the high mortality ratio of the insect after exposure to the powder for 7 days up to 100% to 12 days after the insect was exposed to the Cubeb powder. Manju *et al.*, (2019) are found that All the twelve botanical powders were significantly effective against *Callosobruchus maculatus* insect, of which mixing of 1% seed powder of *Piper nigrum* resulted in 100% mortality within 12 hr. Ofuya and Osadahun (2005) pointed out that *Piper guineense* and *Capsicum frutescens* powders do not cause 100% mortality of adult *Callosobruchus maculatus* insect, even 24 hours. The overlapping effect of exposure durations and mass fractions of Cubeb powder in the rates of insect mortality ratios, the highest average of mortality ratio reached 100% for mass fraction 4g powder on the 7th day also during the period 8-12 days for all the mass fractions, while the lowest rate of insect mortality

Table 4: *Callosobruchus maculatus* mortality ratios using different quantities of Mint powder on Mung bean seeds (corrected according to Abbote equation).

Exposure duration (days)	Mass fractions of Cinnamon powder (gram)				Average of exposure duration	$LSD_{0.05}$
	0.5	1	2	4		
1	0	0	0	0	0	10.67^{**}
2	0	3.33	0	3.33	1.67	
3	0	14.67	11	11	9.17	
4	8	11.67	20	12.67	13.08	
5	5.67	9.33	5.67	21.33	10.5	
6	0	0	0	8.33	2.08	
7	16.67	0	11	0	6.91	
8	66.67	33.33	33.33	0	33.33	
9	100	100	100	83.33	95.83	
10	100	100	100	100	100	
11	100	100	100	100	100	
12	100	100	100	100	100	
Average of Mass fractions	41.42	39.36	40.08	36.67	$LSD_{0.05}$ Mass fractions * Exposure duration	
$LSD_{0.05}$	6.16^{NS}				21.33^{**}	

Table 5: Average and percentage loss by weight of mung bean seeds of the southern Cowpea beetle *Callosobruchus maculatus* Within 35 days of storage.

Storage period (days)	Weight of seeds (gram)	Seed weight loss (%)
7	49.17	1.66
14	47.44	5.12
21	47.41	5.19
28	47.34	5.31
35	47.19	5.62
$LSD_{0.05}$	0.93^{**}	1.85^{**}

Table 6: Percentage ratios of Mung bean seeds germination treated with vegetable powders and at Mass fraction 4g powder/50g seeds.

Powder	Percent of germination (%)
Cinnamon	87.5
Cubeb	85
Mint	77.5
Control	82.5
$LSD_{0.05}$	13.53^{NS}

ratio was 0% for the mass fractions 0.5 and 1g powder during the first day and the mass fractions 0.5, 1and 2g powder during the sixth day of exposure to the powder. Swella and Mushobozy (2007) refer that Black pepper (*Piper* spp.) powder and coconut oil showed good potential in protecting cowpea seeds against bruchid damage.

3. Effect of tested of Mint powder on mortality ratios of the southern Cowpea beetle *Callosobruchus maculatus*

The results of statistical analysis table 4 Confirmed that there was no significant differences in the mass fractions of Mint powder, there were a significant differences for the effect of exposure duration and overlap between mass fractions and exposure duration on average of insect mortality among the Mung Bean seeds. For the exposure periods, the highest mortality ratio of the insect was 100% for the period 10-12 days after the insect was exposed to the Mint powder, while the lowest mortality ratio of insect was 0% after one day. It was cleared from the results as increasing exposure durations the insect to the powder, as increasing the rate of mortality ratio of the insect. Vojoudi *et al.,* (2014) showed that *Mentha pulegium* was more effective essential oil against *Callosobruchus maculatus* beetle compared with the *Zingiber ofûcinale*. Khani and Asghari (2012) pointed out that the oils of *Achillea wilhelmsii* and *Mentha longifolia* have strong insecticidal activity against *Tribolium castaneum* (LC_{50} = 10.02 and 13.05 µl/L air, respectively). The overlapping effect of exposure durations and mass fractions of Mint powder on the rates of insect mortality ratios, the highest rate of mortality ratio reached 100% for mass fraction of 0.5, 1 and 2g powder during 9th day of exposure to the powder and during 10-12 day for all the mass fractions of Mint powder, while the lowest rate of insect mortality ratio was 0% for the mass fractions 0.5g powder during 1, 2, 3 and 6 days of exposure insect to the powder and mass fraction of 1g powder during 1, 6 and 7 days and mass fraction of 1g powder during 1, 6 and 7 days and the mass fraction 2g powder during 1, 7 and 8 days. The deadly effect of peppermint powder may be due to the effect of some compounds on mint leaves. Saeidi and Mirfakhraie (2017) found that the major compounds of *Mentha piperita* oil were menthone (28.9%), menthol (28.5%), and pulegone (6.9%) also LC_{50} of *Mentha piperita* essential oil was 25.70 µL/L air and mortality increased as the doses of essential oil increased. Tandorost and Karimipour (2012) refer that The essential oils Mint species comprise of metabolic compositions such as menhone and menthol compositions and include toxic effects against stored product pests.

Estimation of the percentage loss of the weight of the Mung Bean seeds of the southern Cowpea beetle *Callosobruchus maculatus*

The results show table 5 that the significant effect of weight average of seeds from side and percentage average of seed weight loss and Storage period from other side for Mung Bean seeds that infestation with *Callosobruchus maculatus* during different time periods of storage. The highest average of weight of the Mung Bean seeds reported 49.17g, which was accompanied by the lowest average of percentage loss in the weight of seeds and reached 1.66% after a period of 7 days of storage, While the lowest average weight of the Mung Bean seeds 47.19g, which was accompanied by the highest average of percentage loss in the weight of seeds reached 5.62% after a period 35 of days of storage. Al-Azawi and Mahdi (1983) were pointed out one larva of the Southern Cowpea beetle *Callosobruchus maculatus* consumes about 5% of the weight of the seed, while Belko (1994) states that the cowpea infected with the insect can lose more than 50% of its weight after nine months of storage, and Aylan (2014) founds Powders have the effect of reducing the weight loss of cowpea seeds, the best being clove powder *Syzygium aromaticum* and black pepper powder *Piper nigram* are 2.47% and 2.56% respectively, and the least effect of harmal powder *Peganum harmala* is 6.39%. It was noted from the above results there was a direct increase in the average of percentage loss of weight of Mung Bean seeds increased storage time, this was due to feeding the larvae of southern Cowpea beetle *Callosobruchus maculatus* into the seed. Al-Jassany (2007) mentions that the weight loss percentage of cowpea seeds infected with the southern Cowpea beetle *Callosobruchus maculatus* was 15.55% after 42 days of infection.

Effect of plant powders on the percentage of Mung Bean seeds germination for highest Mass fraction only (4grm powder)

The results table 6 showed that there were no significant differences in averages of germination ratio of Mung Bean seeds treated with vegetable powders (Mass fraction 4g powder/50g seeds). The results showed that there was no effect of vegetable powders on germination ratio of Mung Bean seeds.

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