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Behaviour control of *Callosobruchus maculatus* (F.) using flour and bran of wheat grain

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Abstract

Cowpea seeds is a significant crop for farmers in the world. The cowpea beetle, *Callosobruchus maculatus* is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram and black gram. The weight losses of pulses seeds may reach 800 g/kg in a few months. The excessive reliance on the chemical pesticides only is now under increasing restrictions because of the toxicity hazards to non-target organisms including users, the development of genetically resistant strains of insects, the high cost of application and the environmental impacts. Alternative strategies for the control of insect pests are needed to avoid the hazard associated with the chemical control of pests. As a potential solution to use chemical insecticides, the present study was conducted to evaluate bran and flour of wheat grain as natural agent which affect the behavior of *C. maculatus* beetle. Three bioassay methods were used, viz before insect infestation (protective method), after insect infestation (curative method) as well as repellent activity. The results indicated that the protective method of wheat flour was the best where it reduced the eggs laying and increased the reduction of progeny between 17.22 to 52.32% with the protective method compared to 0.67-19.07% with the curative one. Also, the two methods had the same trend with the wheat bran where the % reduction of F1 progeny ranged from 15.23% - 47.02% with protective method compared to 3.97-22.51% with curative one at the all tested concentrations. In addition that wheat flour had the highest repellent effect in multichoice bioassay (multirepellent) and oppositely the wheat bran had repellent activity more than that of wheat flour in binary choice bioassay (binary repellent). The present study can be recommended to utilize both bran and flour of wheat grain involving integrated pest management of leguminous seed insects since they have many advantages such as more safe, unhazardous for users and environment, cheap, easy available and easy washing.

Keywords: cowpea seeds, C. maculatus, wheat flour, bran flour, repellency.

Introduction

Cowpea grain is infested both in the field and in storage by insect pests. The cowpea weevil, *Callosobruchus maculatus*, Fab. (Coleoptera: Bruchidae) is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram, and black gram (**Talukder and Howse, 1994; Okonkwo and Okoye, 1996; and Park *et al*., 2003).** Unsubstantiated estimates claim that 30% weight loss is due to the infestation of legume seeds by weevils in Africa (**Rodrigues Macedo *et al*., 2000).** Also, heavy infestation of *C. maculatus*¸ cause quality loss, mold growth and impairment of germination in the damaged seeds.

Many methods have been used to prevent these post-harvest losses. Control of stored product insects is best achieved through an integration of physical, chemical and biological methods (**Arthur, 1996; Hagstrum *et al*., 1999; Phillips and Throne, 2010**). But the excessive reliance on the chemical pesticides only is now under increasing restrictions because of the hazards to non-target organisms including users, the development of genetically resistant strains, the high cost of application and the environment impacts (**Bell and Wilson, 1995; Bughio and Wilkins, 2004; Boyer *et al*., 2012).** These concerns have resulted in an increasing attention to alternative strategies for the control of insect pests to avoid the negative drawbacks associated with the chemical control of pests.

Therefore, there is a need to look for alternative organic sources that are readily available, cheap, affordable, relatively less poisonous and less detrimental to the environment (**Udo, 2005).**

As a potential solution to use chemical pesticides, the present study was conducted to evaluate flour and bran of wheat grains as an aspect of green pesticides for grain protecting against stored product insect, *Callosobruchus maculatus* (F.) using repellency and mixing with feeding medium bioassay techniques in laboratory (before and after infestation). Also, the effects of this bioagents on progeny production of the so called pest were evaluated, to our best knowledge, this is the first paper which evaluate the potential of this materials as a factor affecting the behavior control of *C. maculatus* beetle.

Materials and Methods

The tested insect cowpea beetle, *Callosobruchus maculatus* (L.):

Samples of cowpea seeds were obtained from local markets sieved and cleaned from dusts and inert materials. The cowpea seeds were placed in glass jar and sterilized by heating at 70ºC for one hour. The seeds were left to cool and reabsorb moisture. The sterilized seeds were distributed into other jars (500 mL). Each jar was provided with 300-400 adults of

C. maculatus (0-2 day-old) for laying eggs and covered with muslin by rubber band to prevent insect escape. The jars containing insects were incubated at 28+2ºC and 70+5 R.H. for one week. Then the parent adults were sieved out and discarded. Newly adult insects (0-2 day-old) were used for the next experiment:

Materials:
- Flour of wheat grain
- Bran of wheat grain

Methods:
- Before insect infestation
- After insect infestation
- Repellent bioassay

Flour and Bran of wheat:

Wheat grain cleaned, sterilized, and then were milled and sieved. The flour and bran obtained were admixed with cowpea seeds at the concentrations of 1, 2, 3, and 4 g (flour or bran) per 10 g seeds.

Mixing of feeding medium bioassay:

In this experiment, seeds of cowpea were mixed with flour or bran of wheat by two manners (before and after insect infestation).

Before insect infestation:

Cleaned and sterilized cowpea seeds were admixed with flour or bran of wheat at concentrations of 1, 2, 3 and 4 g/10 g seeds. Treated seeds were transferred in Petri dishes (9 cm diameter) and then adult insects of cowpea beetles (*C. maculatus*) were exposed to treated seeds. After 9 days of exposure adult insects were discarded and after 30 days, the number of eggs laying, adult emergence and % reduction of F1 progeny were calculated.

After insect infestation:

Batches of 10 g cleaned and sterilized cowpea seeds were infested with 10 adult insects of *Callosobruchus maculatus* for 10 days for laying eggs, then adults were discarded, the seeds with immature stages were admixed with flour and bran and the emerged adults and % reduction of F1 progeny were calculated according to the following equation:

$$\% \text{ reduction} = \frac{\text{Mean No. of adults of control} - \text{Mean No. of adults of treated}}{\text{Mean No.of adults of control}} x100$$

Repellency bioassay:

Repellency of wheat-derived flour and bran was assessed using dual-choice and multiple-choice bioassay. In dual choice bioassay, portion of 10 g cowpea seeds were mixed thoroughly with 1, 2, 3 and 4 g flour or bran. In multiple choice bioassay, two portions of 10 g cowpea seeds were mixed with flour and bran at 1, 2, 3 and 4 g flour or bran.

Dual choice bioassay:

In this experiment, two Petri dishes (6 cm diameter x 1 cm height) were used. The first was filled with cowpea seeds mixed with flour or bran and the second one was filled with untreated cowpea seeds. The Petri dishes with flour and untreated were placed inside big Petri dishes (12 cm diameter x 2.5 cm height). 20 adult insects (0-2 day-old) were introduced in the center of the big Petri dish and covered with glass lid. The experiment was repeated three times. Repellency was examined after 48 hours according to the following equation:

$$\% \text{ Repellent} = \frac{NC-NT}{NC+NT} x100$$

Where:

NC = The mean number of beetles present in the control chamber.

NT = The mean number of beetles present in the treated chamber

Multiple choice bioassay:

In this experiment, glass jar of 30 x 30 x 10 cm was used as preference chamber comprised three Petri-dishes filled with cowpea seeds treated with flour, bran and the third was untreated. 30 adult insects were introduced in the center of glass jar and covered with glass lid. The experiment was treated three times. Repellency was calculated after 48 h according to the same equation mentioned above.

Results and Discussion

Effect on *C. maculatus* adults (before infestation):

In order to evaluate the effect of wheat flour and wheat bran on the development of *C. maculatus* adults three bioassay methods were used. The first is before insect infestation (protective method), the second is after insect infestation (curative method) as well as the repellent activity of the two wheat derived powders.

Effect of wheat flour:

Protective method (before insect infestation) (effect on adults).

In this experiment, batches of 10 adult insects of *C. maculatus* exposed to 10 g cowpea seeds priorly treated with rates of wheat flour of 1, 2.3 and 4 gm. Results obtained in (Table1) indicated that the all concentrations significantly reduced the number of eggs laying compared to that of untreated control treatment. Also, the concentrations used significantly inhibited the number of hatched eggs. The hatching percentages ranged from 81.9% with the highest concentration to 97.7% with the lowest concentration compared to 100% hatching in control.

In addition, the number of emerged adults significantly influenced at the all tested concentrations, where the percent of progeny reduction ranged from 17.22% to 52.32% in relation to the emerged number of control.

Table 1 . Biology effects of wheat flour admixed with cowpea seeds on *C. maculatus* before insect infestation at indicated concentrations.

Concentration (g)	Mean no. of laying eggs	Mean no. of hatched eggs	% hatchability	Mean no. of emerged adults	% reduction
1	213.0 b	208.0 b	97.7 b	208.33	17.22
2	191.6 c	185.0 c	96.6 c	185.00	26.49
3	168.3 d	155.0 d	92.1 a	155.00	38.41
4	146.6 e	120.0 e	81.9 e	120.00	52.32
Control	251.6 a	251.6 a	100.0 a	251.66`	

In the same column, means followed by the same letter are not significantly different at 0.05 level of significancy according to SPSS.

Curative method (after insect infestation) (effect on immature stages):

In his experiment, adults of *C. maculatus* were allowed to lay eggs then the infested seeds of cowpea were treated by the same concentration of wheat flour (1, 2, 3 and 4 g/10 g) cowpea seeds. The results showed that except, the lowest concentration of 1 g/10 g seeds, the other remained concentrations caused significant inhibition in the percent of hatching. There was slightly effect on the emerged adults of *C. macuatus* with percent of progeny reduction ranged from 0.67 – 19.07% with the all tested concentrations of wheat flour from 1-4 g/10 g seeds. Results presented in Tables 1 and 2 stated that the protective method was the best, where it reduced the eggs laying and increased the reduction of progeny compared to the curative method. For example, the percent of reduction ranged from 17.22 to 52.32% with the first method compared to 0.67-19.07% percent of reduction with the second one.

Table 2. Biology effects of wheat flour admixed with cowpea seeds on *C. maculatus* after insect infestation at indicated concentrations.

Concentration (g)	Mean no. of laying eggs	Mean no. of hatched eggs	% hatchability	Mean no. of emerged adults	% reduction
1	253.3 a	250.0 ab	98.7	250.00	0.67
2	253.3 a	241.0 b	95.1	241.66	3.97
3	251.6 a	225.0 c	89.4	225.00	10.59
4	251.6 a	203.0 d	80.7	203.33	19.07
Control	253.3 a	253.0 a	100	251.66	

In the same column, means followed by the same letter are not significantly different at 0.05 level of significancy according to SPSS.

Effect of wheat bran:

Before infestation:

Similarly, (Table3) included the effect of wheat bran on the development of *C. maculatus* which exposed to cowpea seeds priorly admixed with wheat bran at concentration values of 1, 2, 3 and 4 g/10g seeds. Results accentuated that the all concentrations used significantly reduced the percent of hatching ranged from 93.0 to 97.7% with the all tested concentrations in comparison with control which gave 100% hatching. Meanwhile, the tested rates of wheat bran significantly inhibited the emerged adults with reduction in progeny ranged from 15.23 to 47.02.

Table 3. Biology effects of wheat bran admixed with cowpea seeds on *C. maculatus* before insect infestation at indicated concentrations.

Concentration (g)	Mean no. of laying eggs	Mean no. of hatched eggs	% hatchability	Mean no. of emerged adults	% reduction
1	218.3 b	213.3 b	97.7	213.33	15.23
2	190.0 c	181.6 c	95.6	181.67	27.81
3	166.6 d	156.6 d	94.0	156.67	37.75
4	143.3 e	133.3 e	93.0	133.33	47.02
Control	251.6 a	251.6 a	100.0	251.66	

In the same column, means followed by the same letter are not significantly different at 0.05 level of significancy according to SPSS.

After infestation:

According to the results in Table (4), the same trend of wheat flour effect after infestation was found with the wheat bran where the % hatching significantly influenced by the all concentrations except that of 1 g/10 g seeds. Also, the % reduction in F1 progeny significantly reduced ranging from 3.97-22.51.

Table 4. Biology effects of wheat bran admixed with cowpea seeds on *C. maculatus* after infestation at indicated concentration.

Concentration (g)	Mean no. of laying eggs	Mean no. of hatched eggs	% hatchability	Mean no. of emerged adults	% reduction
1	253.3 a	241.6 ab	95.38	241.67	3.97
2	253.3 a	235.0 b	92.78	235.00	6.49
3	251.6 a	213.3 c	84.78	213.33	15.23
4	251.6 a	195.0 d	77.50	195.00	22.51
Control	253.3 a	253.3 a	100	251.66	

In the same column, means followed by the same letter are not significantly different at 0.05 level of significancy according to SPSS.

Repellent activity:

Two choice bioassay methods were used to evaluate the repellent potency of flour and bran of wheat grain admixed with cowpea seeds against *C. maculatus* adults. The first was dual choice bioassay in which the prefer ability was between one material (flour or bran) and control (cowpea seeds only), while the second was multiple-choice between two materials (flour and bran) and control.

Dual choice bioassay:

In this experiment, the prefer ability was between one material (flour or bran of wheat) and control treatment. Results in Table (5) demonstrated that both bran or flour admixed with cowpea seeds induced gradually repellency according to the increasing of concentrations from 1-4 g/10 g grain after 48 h of exposure to treated medium. The percent of repellency ranged from 46.7 to 90% at the concentrations from 1-4 g with flour, while bran caused repellency percentages between 52.3 to 95.3% presenting the distinction of bran as repellent agent compared to flour when admixed with cowpea seeds in separated experiments.

Table 5. Binary repellent activity of wheat flour and bran admixed with cowpea seeds *C. maculatus* adult at indicated concentration after 48 hours.

Material	Conc. g/10 g seeds				Designing of choice
	1	2	3	4	
Flour	46.7	65.6	75.0	90.0	Binary repellent flour and control
Bran	52.3	78.5	84.5	95.3	Binary repellent bran and control

Multiple choice bioassay (multi-repellent):

In this experiment, the prefer ability conducted between three treatments, two of them were flour and bran of wheat grain, while the third included the control treatment. Data presented in Table (6) showed that flour achieved percent of repellency exceed that of bran, where the repellent activity values were 38.5 to 65.3 for flour and ranged from 19.1 – 48.6% with bran showing that bran had the lowest repellent effect compared to flour at the all tested concentrations (1-4 g) after 48 hours of treatment.

Table 6. Multi repellent activity of wheat flour and bran admixed with cowpea seeds *C. maculatus* adult at indicated concentration after 48 h

Material	Conc. g/10 g seeds				Designing of choice
	1	2	3	4	
Flour	38.5	54.3	57.3	65.3	Multi repellent
Bran	19.1	29.6	44.7	48.6	Flour, bran and control

Discussion

For evaluating wheat derived flour and bran as physical measure belong to green pesticides, three laboratory experiments were set up. The first was admixed flour and bran with cowpea seeds through two manners, before insect infestation (protective method), after insect infestation (curative one) and the third was conducted to determine the repellent activity against adult of cowpea beetle, *C. maculatus.* Data obtained, demonstrated that admixing of flour and bran with cowpea seeds significantly reduced the eggs laying, the % hatchability, the number of emerged adults and the percent of reduction in F1 progeny. In addition that, the flour and bran showed repellent activity against *C. maculatus* beetle after 48 h post treatment.

In general, when mixing flour or bran with cowpea seeds, the chemical substances of cowpea seed which carry information's cannot receive to the cowpea beetles. So, the all tested beetles do not arrive to the host to lay their eggs. Consequently, the total number of eggs laying by insects significantly reduced causing high percent of reduction in the emerged adults. Also, possibly the flour or bran of

wheat grain contain some chemicals which affect the olfactory sensory of cowpea beetles which prevented the beetles to recognize to its hosts either by smell or sight.

In seeking the host, usually insects start moving randomly until they find a particular direction by smell, or sight, affected by the interaction of many different physical, chemical and biotic factors in their environment. These include light intensity, temperature, relative humidity, chemicals that mediate interactions between organisms are called semiochemicals, these include oviposition deterrents, sex and aggregation pheromones, produced by the insects themselves and chemicals associated with their food supply including fungal volatiles. Chemicals deliberately applied to the grain for pest control can also affect insect behavior.

ea fractions (protein, fibre and starch) were mixed thoroughly with Canada Western Hard Red Spring wheat at concentrations of 0, 0.001, 0.01, 0.1, 1.0 and 10.0% (w/w). Food preference chambers (**Loschiavo, 1952**) were used to conduct multiple-choice bioassay. Unsexed adults of each species (*T. castaneum, T. confusum, C. ferrugienus, Rhyzopertha dominica* (F.), *S. oryzae* and *S. zeamais*) were used. Each species was tested separately. We presumed that this reduction was caused by chemosensory effects of the fractions, either olfactory or gustatory. Peas (**Combs *et al.*, 1977; Holloway, 1986; Grenier *et al.*, 1997**). Pea fractions (**Bodnaryk *et al.*, 1999**) and proteins purified from peas (**Deloble *et al.*, 1999**) can be toxic to stored product insects.

Some beetles may craw from outside, invading particularly from neighboring stores containing infested commodities, but also sometimes from rubbish heaps, perhaps attracted by the smell of the new grain **(Southwood and Johnson, 1957; However, 1965a; Barrer, 1983; Throne and Cline, 1994; Delobel and Grenier, 1993)**.

In general, assimilation efficiencies for storage beetles are higher than those of leaf-feeding species while relative growth rates and growth efficiencies reflect the quality of food consumed (**Slansky and Scriber, 1985**).

Some attempts have been made to study the resistance of different varieties of wheat to attack by storage beetles, including *O. surinmensis* (Sinha, 1971). Differences in susceptibility have been attributed to chemical factors such as sterol and alpha-amylase inhibitor levels or physical properties including thickness of the bran layer and hardness of the endosperm (**Sarin and Sharma, 1979; Yetter *et al.*, 1979; Sudhakar and Pandey, 1982; a,b; Gatehouse *et al.*, 1986**). Larval mortality was loosely associated with bran thickness and gram size. However, varietal differences were considered to be of limited practical significance as far as resistance to attack by *O. surinamensis* was concerned.

Other nutrients found in grain, such as maltose and amylopectien, also act as feeding stimulants for storage beetles, including *S. oryzae*, while benzaldehyde from brewer's yeast is attractive to *Oryzaephilus* spp. (**Loschiavo, 1965; Chippendal, 1972; Pierce *et al.*, 1981**).

Conclusion

Flour and bran of wheat showed obviously deterrent effects against *C. maculatus* with the all tested parameters, eggs laying, hatchability, emerged adult, % reduction in F1 progeny beside the repellent activity. These concerns present that the materials used in the current study may exploite as alternatives to chemical insecticides in protecting cowpea seeds. They are good solution where they have many advantages, more safe, easy available and easy removing.

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المكافحة السلوكية لحشرة خنفساء اللوبيا بإستخدام دقيق وردة حبوب القمح

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ملخص البحث

تعتبر بذور اللوبيا من المحاصيل الهامة بالنسبة للمزارعين فى العالم. وتعتبر حشرة خنفساء اللوبيا من الآفات الرئيسية الهامة اقتصاديا لبذور البقوليات مثل اللوبيا والعدس والبسلة وتصل نسبة الفقد فى الوزن إلى 800 جرام لكل كجم فى أشهر قليلة وقد أدى الاعتماد المتزايد على المبيدات الكيماوية فقط فى المكافحة إلى ظهور أخطار للسمية على الكائنات غير المستهدفة ومنها المستخدمين ، كذلك أدى إلى تطور ظاهرة المقاومة وراثيا بالإضافة إلى ارتفاع تكاليف المكافحة وكذلك الأضرار الناجمة على البيئة مما أدى إلى وجود محاذير فى استخدام هذه المبيدات ولتحاشى هذه الأخطار فقد اتجهت الأنظار إلى وجود استراتيجيات بديلة لتحاشى السلبيات المرتبطة بالمكافحة الكيماوية للآفات.

أجريت هذه الدراسة لتقييم كفاءة دقيق وردة حبوب القمح كمواد طبيعية وكأحد الحلول التى تؤثر على سلوك حشرة خنفساء اللوبيا وأيضا كبديل للمبيدات. واستخدمت ثلاث طرق للتقييم الحيوى وهى:

الطريقة الأولى: الطريقة الوقائية وهى خلط الحبوب بالمواد المختبرة قبل الاصابة بحشرة خنفساء اللوبيا.

الطريقة الثانية: الطريقة العلاجية وهى خلط بذور اللوبيا بذات المواد السابقة بعد الاصابة

الطريقة الثالثة: وهى دراسة النشاط الطارد للمواد المختبرة

وقد أشارت النتائج المتحصل عليها أن الطريقة الأولى الوقائية باستخدام دقيق القمح هى الطريقة الأفضل حيث أدت إلى خفض عدد البيض الموضوع وكذلك أدت إلى زيادة الخفض فى التعداد من 17.22 % إلى 52.32% مقارنة 0.67% إلى 19.07% مع الطريقة العلاجية.

أيضا أظهر استخدام الردة نفس الاتجاه الحاصل مع الدقيق كذلك فى كلا الحالتين الوقائية والعلاجية حيث تراوحت نسبة الخفض فى التعداد فى الجيل الأول باستخدام الردة من 15.23% إلى 47.02% مع الطريقة الوقائية مقارنة 3.97% إلى 22.51% مع الطريقة العلاجية وذلك مع جميع التركيزات المستخدمة.

أظهرت النتائج بالاضافة إلى ما سبق أن دقيق القمح كان له التأثير الأعلى أو الأقوى مع طريقة النشاط الطارد المتعدد عكس تأثير ردة القمح التى أدت إلى التأثير الأقوى فى حالة الاختبار الثانى.

توصى هذه الدراسة بإمكانية استغلال كل من دقيق وردة حبوب القمح فى برامج المكافحة المتكاملة للحشرات التى تصيب بذور البقوليات وخصوصا بذور اللوبيا وذلك لمميزاتها العديدة مثل الآمان وعدم خطورتها على المستخدمين والبيئة وكذلك رخص ثمنها وسهولة الحصول عليها وسهولة غسلها والتخلص منها.