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A New Approach in Cowpea Beetle, *Callosobruchus maculatus* (F.) Control in the Light of Climatic Changes

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ABSTRACT

Cowpea, *Vigna unguiculata* is used as human food in many parts of the world, especially in tropical and subtropical regions. The major insect pests that can cause economic loss are the cowpea beetle, *Callosobruchus maculatus*. To promote sustainable development, overcome climate change and minimize chemical insecticide use, evaluation of chemical insecticide (malathion) alone or combined with Gamma radiation (20Gy) was used to control cowpea beetle occurred. Radiation was applied before the chemical treatment. The results indicate there is no different effect of using insecticide alone or insecticide combined with Gamma radiation in parents, while in F1 the results indicated that there is a significant difference within days after treatment. Different concentrations of insecticide were used as well as different methods of treatment (P. value was 0.000 in all of them). Generally, when comparing means of all treatment types with the non-radiated treatment of F1, we can arrange the type of treatment according to potency as the following: an insecticide with the radiated treatment of F1 >insecticide with non-radiated and radiated of parents > radiated treatment only of parent> insecticide only of F1. Thus, the author recommended using of insecticide and Gamma rays' combination in cowpea beetle's control.

INTRODUCTION

Cowpea, (*Vigna unguiculata* (L) Walpers, Fabaceae), is used as human food in many parts of the world especially in tropical and subtropical regions, due to its high protein content (Diouf, 2011). Production of cowpea in Egypt is about 900 kg/feddan. Cowpea is an annual herbaceous legume with trifoliate leaves, as well as it is adapted to the drier regions whereas the other legumes plants do not (Singh, 1987). As well as cowpea plants require rainfall of about 750–1100 mm annually (Skerm *et al*., 1988). An economic loss of cowpea is due to infestation and infection by many insect pests as well as some diseases (Singh and Emden, 1979). The major insect pests that can cause economic loss is cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), which is the principal post-harvesting pest of cowpea crop in the tropics (Caswel, 1981). Cowpea

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weevil pests also can grow inside about 14 Leguminosae-type seeds (Delobel and Tran, 1993). Also, it causes qualitative and quantitative losses of seeds by making seed perforations and reductions in weight, as well as a reduction in market value and germination ability of seeds (Oluwafemi, 2012). The unfixed environment nature of the stored seed causes an infestation of seeds with bruchid beetles. The important pest of seed legumes worldwide is the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), such species is spread throughout the tropical and subtropical (West African origin) (Southgate, 1978). To reduce the high losses of cowpea seeds during storage, it was found that, various control methods and techniques have been developed as well as some more are still under development. Meanwhile, Chemicals pesticide using has a significant impact on public health causing and environmental problems because of their frequent use in agricultural settings to control pests (Mutengwe *et al*., 2016).

Heavy application and indiscriminate applications of insect chemicals as pesticides in the management of pests will advance resistance in all classes of insecticides against insects (Kranthi *et al*., 2002). Moreover, the combination of the different insecticidal treatments showed and exhibit a dramatic reduction in insects in the field as well as minimized the concentration of the insecticide required to reduce the insect population (Shehawy *et al*., 2019). The use of the sub-sterilizing doses of gamma radiation causes suppression in the F1 progeny and the produced progeny becomes more susceptible to surrounding circumstances and insecticides, (Ramesh *et al*., 2002). (Carpenter *et al*., 2005) announced that F1 sterility could be combined with other different control tools in laboratory and field studies.

The aim of this study is to examine the efficacy of the combination of sub-sterilizing doses of gamma radiation with a chemical insecticide, malathion against Cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) to minimize insect resistance and uses of chemical insecticide in the light of climatic changes.

MATERIALS AND METHODS

The Experimental Site:

All experiments were carried out in laboratories of the plant protection Research institute, ARC, Egypt.

Insects Under Study:

The *Callosobruchus maculatus* culture was obtained from infested cowpea seeds maintained in the Stored Grains and Products Pests Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The culture was reared at 28±2°C and 70±2% relative humidity (RH) in continuous darkness. Adult cowpea beetles reared in plant protection research institute on cowpea seeds. The culture of cowpea beetle was reared by placing 100 adults in two-litter jars full in half with disinfected cowpea seeds. Jars were covered with Muslin cloth to prevent cowpea weevils from escaping.

The mating of these adults of cowpea beetles was allowed under laboratory conditions (25–28 °C and 60%–70% relative humidity) until egg laying, the adults were removed. One day after hatching, these new hatching insects were classified into male and female by the elytral pattern test; males are plain with less distinct spots whereas females are maculated with four elytral spots.

Cowpea Seeds:

The cowpea seeds were checked to ensure that they were not infested by visual observation for the presence of eggs or any suspicious material.

Insecticide

Malathion powder recommended dose of 1g/kg.

Irradiation Source:

Newly emerged *C. maculatus* adults were irradiated with 20Gy (Ibrahim *et al*., 2017) using Gamma cell-40 (Co^{60} irradiation unit) with a dose rate of 1.107kGy/h placed at the National Center for Radiation Research and Technology (NCRRT).

Bioassay on the Insect as Parents:

Different concentrations of malathion used (0, 1, 0.5, 0.25 and 0.125 g/kg) with 20 gm cowpea in small jars. 20 newly irradiated and non-irradiated adults were added to each jar. The jars were covered with muslin cloth for sufficient ventilation, and 3 replicates were done for each conc. And incubated at 28±2°C, 70±2% RH. The mortality was counted till getting 100 % mortality in each group. The no. of progeny (F1) and its reduction % from the different treatments of the bioassay experiment were counted for each conc. and replicate.

Bioassay on the Insect as F1:

Different concentrations of malathion used (0, 1, 0.5, 0.25 and 0.125 g/kg) with 20 gm cowpea in small jars. 20 newly irradiated or treated with malathion at parents' adults' phases were added to each jar. The jars were covered with muslin cloth for sufficient ventilation, and 3 replicates were done for each conc. The jars were incubated at 28±2°C, 70±2% RH. The mortality was counted till getting 100 % mortality in each group. The no. of progeny (F2) and its reduction % from the different treatments of the bioassay experiment were counted for each conc. and replicate.

The Fecundity of Insect (No. of eggs laid/♀):

One pair was placed in a jar containing 5 gm cowpea and kept at 28±2°C, 70±2% RH. for mating. The no. of eggs was counted after 1 week. 3 replicates were performed for each crossing combination as follows:

Irradiated ♂ X Unirradiated ♀
Unirradiated ♂ X Irradiated ♀
Irradiated ♂ X Irradiated ♀
Unirradiated ♂ X Unirradiated ♀ (Control).

Data Collection and Statistical Data Analysis:

Data analyses were conducted through the calculation of the mean, standard deviation, standard error, LC values, and correlation coefficient, and analysis of variance was carried out by SPSS software. LC_{90} values were calculated depending on the accumulative mortality after 2days using a software package Ldp-line copyright by Ehab, M. Bakr, Plant Protection Research Institute, ARC, Giza, Egypt. The data of fecundity were statistically evaluated by analysis of variance (F) followed by the Tukey Pairwise comparisons test to examine the significant differences between the treatments.

RESULTS AND DISCUSSION

Data illustrated in Table (1) showed that the calculation of mortality percentages of *C. maculatus* after treatment with different concentrations of malathion (1 gm/K, 0.5 gm/K, 0.25 gm/K and 0.125 gm/K) alone for 4 days was ranged between 96.7:100, 73.3:100, 46.7:100 and 31.7:88.3% respectively. While the mean of the egg number was 1.3, 2.3, 3.0, 4.6 respectively compared with the control which was 171.

Whereas, the mortality percentages of *C. maculatus* after treatment with 1 gm/K, 0.5 gm/K, 0.25 gm/K and 0.125 gm/K concentrations of malathion combined with Gamma radiation were 100, 76.7, 56.7 and 26.7 respectively after the first day while it was 100% after 4 days. While the mean of the egg number was 1.3, 1.3, 1.6, and 2.5 respectively compared with the control which was 121.6 eggs.

Table 1: Mortality % of insecticide-treated alone and/or sinsecticide-treated combined with Gamma radiation against *C. maculatus* parents**.**

Type of treatment	Concentration	1 day	2^{nd} day	3^{rd} day	4^{th} day	F 1	Reduction%
Normal Parents (non-irradiated)	Control (normal)	0±0	0±0	0±0	0±0	58.3±6.9	-
	1 gm/K	96.7±3.3	100±0	100±0	100±0	0±0	100
	0.5 gm/K	73.3±8.8	100±0	100±0	100±0	0±0	100
	0.25 gm/K	46.7±3.3	100±0	100±0	100±0	0.7±0.3	98.8
	0.125 gm/K	31.7±11.6	76.7±15.9	81.7±15.9	86.7±13.3	58.3±32.4	0
Irradiated Parents	Control	0±0	0±0	0±0	0±0	20.3±	65.2
	1 gm/K	100±0	100±0	100±0	100±0	0±0	100
	0.5 gm/K	56.7±3.3	96.7±3.3	100±0	100±0	0±0	100
	0.25 gm/K	26.7±6.6	90±10	90±10	96.7±3.3	0±0	100
	0.125gm/k	9.6±0	35±5	50±3.3	58±3.3	0±0	100

Data represented in Table (2) showed that there is a significant variance between the concentration which were used in this study (P. value was 0.000). Whereas the same data showed that there is no significant variance between the types of treatment (radiated or noon radiated treatment), the P value was (0.307). Thus, the results indicated that there is a no different effect of using insecticide alone or insecticide combined with Gamma radiation.

Table 2: Analysis of variance between insecticide-treated alone and/or insecticide-treated combined with Gamma radiation against *C. maculatus* parents.

Source	Type III Sum of Squares	D.F.	Mean Square	F	Sig.
Concentrations	27157.491	3	6789.373	13.421	.000
Radiated and non-radiated treatments	535.185	1	535.185	1.058	.307
Error	33386.815	67	505.861		
Total	500081.000	72			
Corrected Total	63124.319	71			
a. R Squared = .471 (Adjusted R Squared = .431)					

Data tabulated in Table (3) showed that, the calculation of mortality percentages of *C. maculatus* (F1) after treatment with different concentrations of malathion (1 gm/K, 0.5 gm/K, 0.25 gm/K and 0.125 gm/K) alone for 4 days. The mortality% was ranged between 98.33:100, 60.0:86.67, 0.0:8.33 and 0.0:0.0 respectively. Whereas, the mortality percentages of *C. maculatus* (F1) after treatment with 1 gm/K, 0.5 gm/K, 0.25 gm/K and 0.125 gm/K concentrations of malathion combined with Gamma radiation were 100, 100, 80 and 48.3 respectively after the first day while it was 100, 100, 100 and 95 respectively after 4 days according to the four-concentration used. Those results are like that of Ivanishvili (2016) an effect of such a strong damaging factor as ionizing radiation.

Table 3: Mortality % of F1 after insecticide alone and insecticide combined with gamma rays at the parent's phase of *C. maculatus*.

Treatment	Concentrations	1 day	2nd days	3rd days	4th days	F2	Reduction%
F1 Treated with malathion 0.025g/k at the parents' phase	Control	0±0	0±0	0±0	0±0	0±0	100
	1 gm/K	98.33±1.6	100±0	100±0	100±0	4.33±	92.57
	0.5 gm/K	60±23	73.33±13.3	86.67±6.6	86.67±6.6	66±13.8	0
	0.25 gm/K	0±0	1.67±1.6	8.33±3.3	8.33±3.3	137±7.2	0
	0.125 gm/K	0±0	0±0	0±0	0±0	157±17	0
F1 Irradiated at the parents' phase	Control	0±0	0±0	3.3±3.3	10±5.7	43±4.7	26.24
	1 gm/K	100±0	100±0	100±0	100±0	0±0	100
	0.5 gm/K	100±0	100±0	100±0	100±0	1.33±0.27	97
	0.25 gm/K	80±2.9	95±2.9	100±0	100±0	2±0	95.35
	0.125 gm/K	48.3±10	81.7±1.6	85±5	95±0	9.66±2.2	77.53

The data represented in Table (4) showed an analysis of the variance of F1 after treatment with insecticide alone or combined with Gamma radiation. The results approved that there is a significant variance between the four days after treatment and the concentration which were used in this study (P. value was 0.000). As well as, the type of treatment (insecticide alone or combined with Gamma radiation), the P value was (0.000). Thus, the results indicated that there is a significant difference between using insecticide alone and insecticide combinations in the first generation. It means that using Gamma radiation is very useful in the control of C. maculatus combined with insecticide. Because using Gamma radiation combined with insecticide is more potent than insecticide alone in the first generation, this may be due to breaking resistance in the first-generation table (4). These results are going in that same line with El-Gizawy (2018) that who reported that the additive effect was more detectable when higher doses of gamma radiation were combined with LC50 of either of the two essential oils.

Table 4: Analysis of variance between insecticide-treated alone and/or insecticide-treated combined with Gamma radiation against F1 *C. maculatus* beetle.

Source	Type III Sum of Squares	D. F.	Mean Square	F	Sig.
Day	1638.361	2	819.181	24.668	.000
Conc	48127.486	3	16042.495	483.086	.000
Type	41136.681	1	41136.681	1238.746	.000
day * conc	663.306	6	110.551	3.329	.008
day * type	250.028	2	125.014	3.765	.030
conc * type	20582.486	3	6860.829	206.600	.000
day * conc * type	1998.306	6	333.051	10.029	.000
Error	1594.000	48	33.208		
Total	438529.000	72			
Corrected Total	115990.653	71			
a. R Squared = .986 (Adjusted R Squared = .980)					

Data analysis and represented in Table 5 showed the analysis of variance between insecticide alone and/or combined with Gamma radiation in parents as well as in the First generation. The results indicated that there is a significant difference within days after treatment, a different concentration which was used in F1 as well as different methods of treatment (P. value was 0.000 in all of them) Table (5).

Table 5: Analysis of variance between Insecticide treated alone and/or insecticide-treated combined with Gamma radiation against parents and F1 *C. maculatus* beetle.

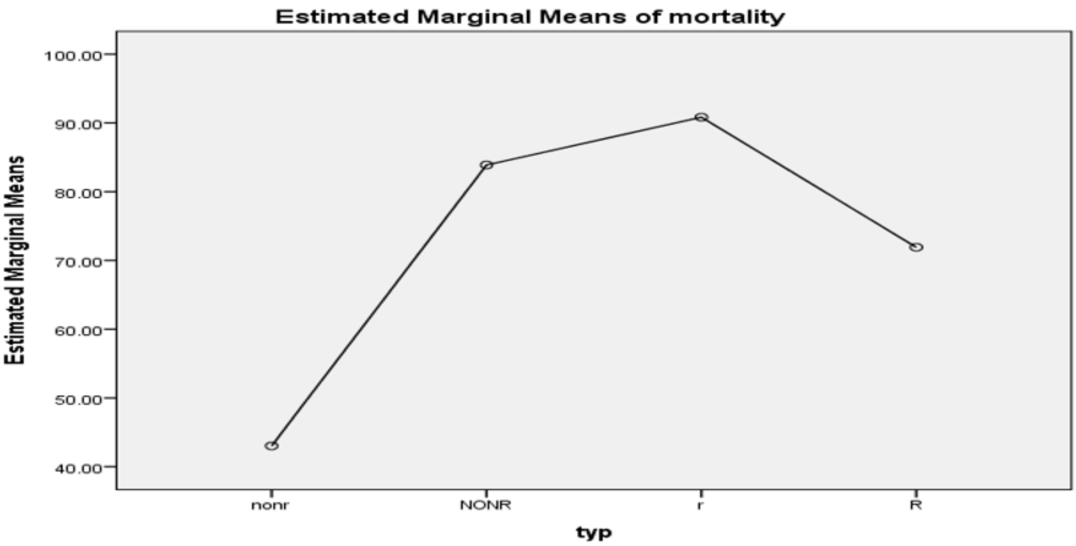
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Days	15329.292	2	7664.646	20.679	.000
Conc	70401.611	3	17600.403	47.485	.000
Typ	48186.750	3	16062.250	43.335	.000
Error	49667.375	134	370.652		
Total	938610.000	143			
Corrected Total	183449.000	142			
a. R Squared = .729 (Adjusted R Squared = .711)					

Data evaluated and represented in Table (6) showed the difference among all types (Insecticide treated alone and/or insecticide combined with Gamma radiation) against parents and F1 *C. maculatus*. According to the least significant difference (LSD) the recorded results indicated that the efficacy of non-radiated and radiated treatment of parent as well as radiated treatment of F1 (Mean Difference was 41.3, 28.88 and 47.80) was more potent than that of non-radiated treatment of F1. Thus, when comparing the means of all treatment types with the non-radiated treatment of F1, we can arrange the type of treatment according to potency as the following: radiated treatment of F1 > non-radiated and radiated of parents > radiated treatment of parent. Meanwhile, when comparing the means of all treatment types with the non-radiated treatment of parents, we can arrange the type of treatment according to potency as the following: radiated treatment of F1 > non-radiated and radiated of parents. Whereas, non-radiated and radiated of parents > radiated treatment of parent and non-radiated and radiated of F1. According to LSD when comparing of radiated treatment efficacy of F1 with the non-radiated treatment of F1, radiated treatment of parents and non-radiated treatment of parents, the results indicated that the radiated treatment efficacy of F1 was highly potent than all of their Table (6) & Fig. (1). The conducted data are in the same line with that of Shishir *et al*., (2009) who concluded that Gamma radiations are used to produce mortality or sterility in the insects. This technique can be used by irradiating the insects at doses sufficiently high to produce the desired effects.

Table 6: LSD difference between insecticide-treated alone and/or insecticide-treated combined with Gamma radiation against parents and F1 of *C. maculatus* beetle.

	(I) typ	(J) typ	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	nonr	NONR	-41.2778*	2.08028	.000	-45.4071	-37.1485
		R	-47.8056*	2.08028	.000	-51.9349	-43.6762
		R	-28.8889*	2.08028	.000	-33.0182	-24.7596
	NONR	nonr	41.2778*	2.08028	.000	37.1485	45.4071
		R	-6.5278*	2.08028	.002	-10.6571	-2.3985
		R	12.3889*	2.08028	.000	8.2596	16.5182
	R	nonr	47.8056*	2.08028	.000	43.6762	51.9349
		NONR	6.5278*	2.08028	.002	2.3985	10.6571
		R	18.9167*	2.08028	.000	14.7874	23.0460
	R	nonr	28.8889*	2.08028	.000	24.7596	33.0182
		NONR	-12.3889*	2.08028	.000	-16.5182	-8.2596
		R	-18.9167*	2.08028	.000	-23.0460	-14.7874

R= Efficacy of radiated treatment in parents, r= = Efficacy of radiated treatment in F1, nonr=insecticide treatment alone in F1 and NONR= insecticide treatment alone in parents.



R= Efficacy of radiated treatment in parents, r= = Efficacy of radiated treatment in F1, nonr=insecticide treatment alone in F1 and NONR= insecticide treatment alone in parents.

Fig 1. Estimated marginal means of mortality % against parents and F1 of *C. maculatus*. Where, R= radiated in parents, r= F1 radiated treatment in parents' phase, nonr=insecticide treatment in parents and F1 and NONR= insecticide treatment in parents.

Data presented in Table (7) showed the number of eggs after treatment compared with that of control, the mean number of eggs in unirradiated males and females was 52, while it was 45.33, 35.67 and 18 for irradiated ♂ X unirradiated ♀, unirradiated ♂ X irradiated ♀ and irradiated ♂ X irradiated ♀ respectively. It means that there is no significant difference between control and irradiated ♂ X unirradiated ♀. While there was a significant difference between unirradiated ♂ X irradiated ♀, irradiated ♂ X irradiated ♀ and unirradiated ♂ X unirradiated ♀ (Control). By the results, we can conclude that the number of eggs produced is affected by radiation in a reverse way. These results are in agreement with that of Mohapatra and Giri (2015) who mentioned that in order to control pests, we can sterilize the pests by ionizing radiation (at lower doses) and release it into the infested area to mate Table (7).

Table 7: Fecundity of irradiated adult of *C. maculatus* beetle.

Groups	No. of eggs after 1 week
Unirradiated ♂ X Unirradiated ♀ (Control)	52±1.5^{a}
Irradiated ♂ X Unirradiated ♀	45.33±2.3^{a}
Unirradiated ♂ X Irradiated ♀	35.67±1.67^{b}
Irradiated ♂ X Irradiated ♀	18±2.3^{c}

In general, the data revealed are in the same line with Ahmadi *et al.*, (2013) when combined radiation with essential oils on *Tribolium castanum* and stated that gamma radiation with essential oils made a synergistic effect also could be used as combined methods in IPM.

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ARABIC SUMMARY

نهج جديد لمكافحة خنفساء اللوبيا *Callosobruchus Maculatus* (F.) في ضوء التغيرات المناخية

أيمن على شهاوى[1] - نيللى أحمد حسن عبد الفتاح[2] - رحاب محمد سيد[3]

1- معمل المكافحة الخضراء للآفات - معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - الجيزة - مصر.

2- قسم بحوث افات الحبوب المخزونة ومنتجاتها - عهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - الجيزة - مصر.

3- قسم بحوث المنتجات الطبيعية - المركز القومي لبحوث وتكنولوجيا الاشعاع - هيئة الطاقة الذرية المصرية - القاهرة - مصر.

يستخدم الانسان اللوبيا كغذاء في معظم دول العالم وخاصة في المناطق الاستوائية وشبه الاستوائية. وتعتبر خنفساء اللوبيا (*Callosobruchus maculatus*) من الآفات الحشرية الرئيسية التي يمكن أن تسبب خسائر اقتصادية فادحة. لهذا المحصول المهم لذا فان كميات هائلة من المبيدات تستخدم لمكافحة هذه الافة. وفي هذا السياق وللعمل علي تعزيز التنمية المستدامة وللتغلب على الأثار السلبية للتغيرات المناخية فانه من الواجب علينا تقليل استخدام المبيدات الحشرية الكيميائية. تم في هذه الدراسة تقييم مركب الملاثيون منفردا ثم تقيمه مع إشعة جاما (Gy20) للسيطرة على خنفساء اللوبيا. أشارت النتائج إلى عدم وجود تأثير مختلف لاستخدام المبيد الحشري وحده أو المبيد الحشري مع إشعاع جاما عند الاباء، بينما في F1 أشارت النتائج إلى وجود فرق معنوي في غضون أيام بعد المعاملة المزدوجه (ملاثيون مع الاشعاع). بشكل عام ، عند مقارنة جميع أنواع المعاملات بالمعاملة غير المشعة لـ F1 ، يمكننا ترتيب المعاملات من حيث الكفاءة والفعالية علي مكافحة خنفساء اللوبيا على النحو التالي: الملاثيون مع معالجة إشعاعية لـ F1< ملاثيون غير مشعع للاباء< معالجة إشعاعيه فقط للاباء > ملاثيون غيرمشعع ل F1. لذا فقد أوصى المؤلف باستخدام الملاثيون مع أشعة جاما لمكافحة خنفساء اللوبيا. لتقليل استخدام المبيدات بصفه عامه وللحد من الاثار السلبية للتغيرات المناخية.