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SOME OBSERVATIONS ON THE EFFECTS OF GAMMA RADIATION ON EGGS OF PULSE WEEVILS (COLEOPTERA: BRUCHIDAE)*

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Pulses are an important part of daily diet of communities in South Asia subcontinent. Being rich in proteins pulses are commonly referred to as poor man's meat. Pulses are, however, severly attacked by certain bruchid species of which *Callosobruchus analis* F., *Callosobruchus chinensis* L. and *Callosobruchus maculatus* F. are important. These insects can be controlled by the use of toxic fumigants but there ware certain problems associated with such treatment such as the undesirable residues which may remain on the pulses and the possibility of development of resistance after their repeated use. It has also been observed that egg stages of some species of stored grain pests are not killed by such fumigants if not properly carried out. In many countries of the world, efforts are, therefore, being made to control stored grain pest using radiation as an alternative method. Huque and Khan[1] studied the radiosensitivity of various developmental stages of *Callosobruchus analis* F., (identified by them as *Callosobruchus subinnotatus* Pic.) and reported complete mortality in the egg stage even at the lowest dose of 2.5 krad used by them. They suggested further studies with dose of gamma radiation lower than 2.5 krad so as to establish the radiosensitivity of eggs of *Callosobruchus* spp. The present studies were, therefore, conducted and results obtained are reported here.

Materials and Methods

Insects used in these tests were obtained from the culture maintained in our laboratory at 30°C. Eggs were obtained on chickpeas (*Cicer arientinum*) and were aged 1, 2, 3 and 4 days and irradiated in cobalt-60 source (Gamma cell-200) at 124.56 krad /hr. The number of eggs used per treatment varied from 75–150 according to availability. Larvae obtained from the irradiated eggs were allowed to feed on chickpeas. Control were kept in all cases and were subjected to similar treatment except exposure to irradiation. Three replications were made for each treatment.

Results and Discussions

In all the three species, hatching was observed in eggs irradiated at 0.5 krad though it was significantly less than in controls. Eggs of *Callosobruchus chinensis* seem to be more susceptible to gamma radiation compared with eggs of the other two species. In the case of *Callosobruchus analis* and *Callosobruchus maculatus*, some eggs aged 3 and 4 days before irradiation hatched at 1.0 krad. It can be seen that a dose of 1.5 krad prevented hatching in all the eggs of all age groups of the three species tested (Table 1). Huque and Khan reported significant increase in the incubation period of irradiated eggs and death within 24 hr of all the larvae hatched from irradiated eggs. In our case, however, no such abnormalities were observed in cases where hatching took place out of irradiated eggs. The finding of our result also confirm with those obtained by Nicholas and Wiant[2] with twelve species of stored grain pests and Cornwell[3] with grain and rice weevils that resistance of eggs changes with age. From these results, it is concluded that gamma radiation dose of 1.5 krad is sufficient to prevent hatching in eggs of bruchid species infesting pulses in the subcontinent.

TABLE 1. EFFECT OF GAMMA RADIATION ON THE VIABILITY OF EGGS OF *Callosobruchus analis* F., *Callosobruchus chinensis* L. and *Callosobruchus maculatus* F. and SURVIVAL OF RESULTANT LARVAE TO ADULT STAGE.

Species	Age of eggs (day)	Percentage hatching and adult formation in bracket after irradiation at various doses (Krad)				
		Control	0·5	1·0	1·5	2·0
Callosobruchus analis F.	1	78 (78)	62 (61)	0 (0)	0 (0)	0 (0)
	2	73 (73)	32 (30)	4 (0)	0 (0)	0 (0)
	3	77 (76)	50 (48)	46 (44)	0 (0)	0 (0)
	4	80 (80)	54 (54)	48 (45)	0 (0)	0 (0)
Callosobruchus chinensis L.	1	96 (96)	52 (52)	0 (0)	0 (0)	0 (0)
	2	79 (78)	46 (45)	0 (0)	0 (0)	0 (0)
	3	77 (76)	47 (46)	0 (0)	0 (0)	0 (0)
	4	81 (80)	64 (64)	0 (0)	0 (0)	0 (0)
Callosobruchus maculatus F.	1	80 (80)	48 (48)	0 (0)	0 (0)	0 (0)
	2	78 (76)	43 (42)	0 (0)	0 (0)	0 (0)
	3	86 (84)	49 (48)	10 (6)	0 (0)	0 (0)

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PRODUCTION OF COBALAMIN BY STREPTOMYCES OLIVACEOUS

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Cobalamin (vitamin B_{12}) is produced by fermentation different strains of microorganisms. Aside from bacteria and actinomycetes, yeasts and filamentous fungi produce insignificant amounts of the vitamin. The present work deals with the utilization of certain local raw by-products for the production of cobalamin by *Streptomyces olivaceous*.

Maintenance of Streptomyces olivaceous. The organism was maintained on the following ingredients (g/l): glucose, 20.0; $NaNO_3$, 2.0; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4 . 7H_2O$, 0.5; $FeSO_4 . 7H_2O$, 0.005; agar agar, 20.0 and 1000 ml distilled water. These ingredients were digested and portioned into test tubes and sterilized at 1.2 atmospheric pressure at 120°C for 15 min. When the slants attained room temperature, they were inoculated with *Streptomyces olivaceous* and incubated at 27–30°C for 10 days to obtain luxuriant growth and sporulation. The slants were then kept in a refrigerator at 5°C.

Preparation of Inoculum. The organism was grown on a vegetative medium containing the following ingredients (g/l): starch, 50.0, corn steep liquor, 5.0; $CaCO_3$, 5.0; NaCl, 5.0; NH_4NO_3, 5.0 and $CoCl_2$, 0.0025. The ingredients were thoroughly dissolved in 1000 ml distilled water. The medium was portioned into 250-ml Erlenmeyer flask each containing 50 ml of the medium. The initial pH value of the vegetative medium was adjusted to about 7.0. The flasks were plugged and sterilized at 1.2 atmospheric pressure for 20 min. When the flasks attained room temperature (30°C), they were inoculated with a standard inoculum of spore suspension of *Streptomyces olivaceous* under aseptic conditions. The inoculated flasks were inserted on a rotary shaker (200 rev/min) at 27–30°C for 48 hr. At the end of the incubation period, the fermented vegetative medium was ready to inoculate the fermentation medium.

Fermentation Medium. The medium used contained the following ingredients ((g/l) : corn steep liquor, 5.0 $(NH_4)H_2PO_4$, 2.0; $CaCO_3$, 5.0; NaCl, 2.3; $CoCl_2$ 0.0025 and starch, 50.0. The ingredients were thoroughly dissolved in 1000 ml distilled water. The medium was portioned into 250–ml Erlenmeyer flasks, each containing 50 ml of the fermentation medium. The flasks were plugged and sterilized at 1.2 atomospheric pressure for 20 min. After attaining room temperature, the flasks were inoculated with a standard inoculum of the fermented vegetative medium under sterile conditions. The inoculated flasks were inserted on a rotary shaker (200 rev/min) 27–30°C for different incubation periods. At the end of the incubatiion period, the final pH value of the fermented medium, microbial growth and vitamin were investigated.

Determination of Cobalamin. The amounts of cobalamin produced by *Streptomyces olivaceous* were determined spectrophotometrically using the technique of Rudkin and Taylor.[8]

Nitrogen and Reducing Sugars. Total nitrogen was detremined by the technique of Markham[2] and the reducing sugars were determined by the method of Somogyi.[10]

Treatments of Black Strap Molasses. Egyptian black strap molasses contains appreciable essential, elements necessary for the growth of microorganisms especially carbon, nitrogen, magnesium, calcium and certain trace elements such as manganese, iron, zinc, copper and others. Generally, the presence of high level of salts decrease to some extent its suitability for the prduction of certain valuable microbial products. For improvingt its efficiency, the Egyptian black strap molasses was treated with certain chelating ingredients. Undesirable materials were removed and the black strap molasses was diluted with distilled water (1:2, v/v) and the pH adjusted to 6.5–7.0. The solution was centrifuged and then the clear solution was treated with different concentrations of chelating ingredients (EDTA, methylen blue and potassium ferrocyanide).

Treated molasses was used in the fermentation medium replacing its carbon source as a cheap raw byproduct. Fodder yeast produced by *Saccharomyces cerevisiae* was used as organic nitrogen source replacing corn-steep liquor. Measurements of the pH of the fermentation medium was made before and after the fermentation process.

The carbon and nitrogen sources of the basal fermentation medium were replaced by equivalent amounts of the different treatments of black strap molasses and corn-steep liquor respectively. The total ingredients of the fermentation medium were portioned into 250–ml Erlenmeyer flasks each containing 50 ml, plugged and sterilized at 1.2 atmospheric pressure for 20 min. When the flasks attained room temperature, they were inoculated with a standard inoculum of the vegetative medium of *Streptomyces olivaceous* under aseptic conditions. The inoculated flasks were inserted on a rotary

shaker (200 rev/men) at 27°C for a suitable incubation period. At the end of the fermentation process final pH value, suspended matter including microbial growth and cobalamin (vitamin B_{12}) were determined.

Production of cobalamin by *Streptomyces olivaceous* increased with the increase in the incubation period (Table 1). With increased microbial growth, the amount of cobalamin produced also increased (3.0 μg/ml). The initial pH of the fermentation medium was 7.0, and when the fermentation process proceeded, a slight drop towards acidity was obtained and this may be due to the accumulation of certain acids (Table 1.) When the incubation period was increased the total reducing sugars present in the fermentation medium were decreased due to their utilization for the microbial growth and incorporation into other microbial metabolites including cobalamin. Total nitrogen present in the fermentation medium was also decreased due to its utilization for enzymatic processes of the microorganism. After 24-hr incubation no cobalamin was produced by the microorganism, while with the increase in the incubation period the amounts of vitamin were increased reaching its optimum at the end of the fermentation process (120-hr).

The amount of cobalamin produced increased with the increase in the concentration of black strap molasses, reaching its optimum at 15 g/l (Table 2), above which a slight decrease in the yield of vitamin was recorded. Treatment of Egyptian black strap molasses with different concentrations of EDTA, potassium ferrocyanide and methylene blue did not improve its efficiency.

TABLE 1. COBALAMIN PRODUCED BY *Streptomyces olivaceous* AT DIFFERENT INCUBATION PERIOD OF FERMENTATION.

Incubation period (hr)	Final pH value*	Suspended matter (mg/ml)	Consumed-sugars (mg/ml)	Total nitrogen† (mg/ml)	Cobalamin (g/μml)
24	6·5	5·7	3·4	3·3	nil
48	7·0	6·9	7·0	2·2	1·5
72	8·0	7·8	7·6	1·8	2·2
96	8·5	8·4	7·9	1·5	2.7
120	9·0	9·0	8·1	1·2	3·0

*Initial pH value of the fermentation medium was 7·0

†Initial total nitrogen present in the fermentation medium was 5·7 mg/ml. Total nitrogen was measured in the fermentation broth.

TABLE 2. COBALAMIN PRODUCED BY *Streftomyces olivaceous* GROWN ON MEDIUM CONTAINING DIFFERENT CONCENTRATIONS OF BLACK STRAP MOLASSES.

Egyptian black strap moalsses (mg/ml)	Final pH value	Suspended matter (mg/ml)	Cobalamin (μg/ml)
2·5	8·0	6·1	ni
5·0	8·0	6·8	1·5
7·5	8·0	7·7	2·1
10·0	8·0	8·5	2·7
15·0	8·0	9·2	3·5
20·0	7·0	9·0	3·2
30·0	6·5	8·3	2·5
40·0	6·0	8·3	2·5
50·0	6·0	7·9	2·2

TABLE 3. COBALAMIN PRODUCED BY *Streptomyces olivaceous* GROWN ON MEDIUM CONTAINING DIFFERENT CONCENTRATIONS OF FODDER YEAST AS NITROGEN SOURCE.

Fodder yeast (g/1)	Final pH*	Suspended matter (mg/ml)	Cobalamin (μg/ml)
1	8·0	7·1	1·7
5	8·0	7·5	2·0
10	8·0	8·2	2·4
15	9·0	9·0	2·8
20	9·0	9·0	2·8
25	8·0	8·9	2·7
30	7·5	8·6	2·7

*The initial pH of the fermentation medium was 7·0.

The amounts of cobalamin produced increased with the increase of fodder yeast concentration (Table 3), reaching its optimum at 15–20 g/l, above which a slight decrease in the vitamin titre was obtained.

The fermentative production of cobalamin using black strap molasses and fodder yeast as carbon and nitrogen sources gave satisfactory results. The presence of the nutritive elements in the molasses does not mean that it is present in readily available form for the specified organisms. Addition of complementary nutritive elements might be quite necessary in certain cases. Fodder yeast is produced fermentatively by *Saccharomyces cerevisiae*. The fodder yeast contains protein, 56.0%; digestible protein, 53. 0%; air-moist protein, 52.0%; ash content, 10.0%; thiamin, 38.0 mg/kg; vitamin B_2, 38.0 mg/kg; nicotinic amide, 380.0 mg/kg; B_6, 20.0 mg/kg; pantothenic acid, 5.0 mg/kg and folic acid, 28.0 mg/kg. It also contains Fe 280 p.p.m./100g Mn 98.0 p.p.m./100, g, Zn 200.0 p.p.m./100 g and Cu 50.0 p.p.m./100 g. The chemical constituents of fodder yeast reflect its suitability for the biosynthesis and production of cobalamin by *S. olivaceous*.

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