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USE OF IONIZING RADIATION IN INTERACTION WITH FUMIGANTS TOWARDS MANAGEMENT OF *TRIBOLIUM CASTANEUM* (HERBST)

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ABSTRACT

Effect of the interaction of gamma radiation and four commonly used fumigants (EDCT, ethylene dibromide, carbon disuphide and methyl bromide) on the larvae and adults of *Tribolium castaneum* (Herbst) was determined. Two experimental regimens were investigated, (a) irradiation followed by fumigation, (b) fumigation followed by irradiation. It was noticed that the *T. castaneum* exposed to gamma radiation (50 Gy to larvae; 100 Gy to adults), if fumigated within 6 h after irradiation, required higher doses of fumigants at LC_{50} level, than when fumigation was performed 7 days after irradiation.. On the other hand, the fumigation of *T. castaneum* life stages followed by irradiation did not affect their radio-susceptibility. These studies indicated that if irradiation was done before fumigation, the pest susceptibility towards fumigants might be altered. However, no evident change in the susceptibility pattern was noticed when the survivors of the fumigation treatment were exposed to gamma radiation. Merits and demerits of irradiation-technology are discussed. The findings indicate that the ionizing radiation might be considered as an environmentally compatible alternative or supplement to other control methods for stored product-pests.

INTRODUCTION

Insects continue to remain as one of the most serious hazards to stored grains all over the world. Insect pests inflict their damage on stored products mainly by direct feeding and through contamination by insect parts. In tropical and subtropical climates, the problems posed by stored grain insects are of paramount importance. With a view to reducing losses, various methods of safe storage based on the use of improved storage facilities, prophylactic treatment and fumigation of the commodities have been tried with varying degrees of success. The conventional methods of controlling these insects have varied from age-old sun treatment all the way to modern insecticides or fumigation methods. Fumigation, though by far the most effective method of grain disinfestation, has its own serous limitations.

Fumigants are low molecular weight chemicals, highly toxic and volatile, that are used during storage to kill all insect stages residing in the produce. Fumigation is a widely used method all over the world on small as well as large storage scale. The efficiency of application is governed by the physical factors of distribution and penetration of the fumigant into all parts of the grain. This modality is also subject to human errors as well as various other modifying factors, associated with application hazards and toxicity of residues left behind. The development of resistance among insects through persistent application of chemical insecticides is yet another constraint to opt and devise a better method of grain disinfestation that would be environmentally safe.

Two or more components of pest control can be combined in an integrated program. However, in a storage ecosystem, hygiene and good warehouse management are basic requirements. The combination of two or more of the following practices may constitute an effective pest control program, viz., (i) improved harvesting and threshing techniques; (ii) judicious use of insecticides; (iii) use of ambient and/or refrigerated aeration; (iv) atmospheric gas modification; (v) thermal disinfestation; (vi) irradiation techniques; (vii) insect growth regulators; (viii) biological control; and (ix) use of resistant varieties.

The use of ionizing radiation has been recommended not only as a possible alternative but also as a supplement to other control methods (Cornwell 1966,Watters 1968). Combination of gamma radiation with treatments *viz.,* microwaves, infra-red radiation and insecticides has also been evaluated by various researchers (Cogburn and Mahany 1969, Cogburn et al. 1971, Cogburn and Speirs 1972, Tilton et al. 1972, Kinkade and Erdman 1973, Kirkpatrick et al. 1973, Tilton and Brower 1973). However, information on the combined effect of radiation and fumigation is very limited (Cogburn and Gillenwater 1972, Saxena and Bhatia 1981).

The success of green revolution has enabled India to produce over 160 million tonnes of food grains ever year. However, inadequate storage facilities lead to losses, amounting to 10-15% every year, due to insects alone. With progressive increase in the quantity of food grains and necessity for longer storage periods, these losses will escalate unless disinfestation measures are improved. Chemical disinfestations methods, such as fumigation, require repeated application, as these do not eliminate insect eggs. They may also leave harmful residues in the treated grains. Low dose irradiation completely kills or sterilizes the common grain pests, and even the eggs deposited inside the grains. Moreover, only a single radiation exposure of grains could be sufficient for disinfestations. The irradiation, therefore, is ideally suited for large-scale operations, thereby offering substantial economic benefits. Irradiation can also serve as an effective process for disinfestation of certain pre-packed cereal products.

Several species of *Tribolium* are economically important pests and can be found in almost every store containing infested cereals or cereal products, specially in tropical and sub-tropical climates. *T. castaneum* is a very common and serious pest which attacks maize, wheat, flour and other foodstuffs, in tropical climates (Mills & White, 1994). *T. castaneum* adult females lay small, cylindrical, white eggs scattered in the product. At an optimum temperature of 30-32.5^{0}C, the females are highly fecund. Larvae are yellowish with a pale brown head, and they live inside grains until pupation. Adults are about 3-4 mm long and can live for a year or more. This species is also highly tolerable to humidity. The adults are highly adapted to feed on a very wide range of commodities and perfect colonizers of new habitats. In tropical conditions, this species is dominant to *T. confusum* (Howe, 1962).

Studies were, therefore, undertaken to assess the combined effect of gamma radiation and some of the commonly used fumigants against *Tribolium castaneum* (Herbst).

MATERIALS AND METHODS

Method of rearing the test insect, *Tribolium castaneum* (Herbst) on wheat flour was followed at 30±1ºC and 70±5% relative humidity, as described by Mehta and Sethi (1984). The fumigants tested during the present investigation were: ethylene dibromide-carbon tetrachloride – 3:1 mixture (EDCT); ethylene dibromide (EDB); carbon disulphide (CS_2) and methyl bromide (MB). The test insects were exposed to different concentrations of the fumigants for 24 h in 250 ml, glass conical flasks, fitted with a stopcock. During exposure the insects were confined in vented gelatin capsules. The gases were transferred by a transfer burette. Insects were provided sterilized wheat flour as food during the experiments.

Studies on the combined effect of gamma radiation and fumigants on the larvae and adults of *T. castaneum* were undertaken as follows. In treatment A, where irradiation was followed by fumigation, 10 day old larvae and 8-10 day old adults of *T. castaneum* were exposed to 5 and 10 krad of gamma radiation (close to LD_{50}), respectively. The insects were then treated with different fumigants within 6 h and 7 days after irradiation. Each treatment was replicated thrice with 25 insects per replication. Two sets of control *i.e.,* one with only fumigated insects and another without any treatment, were maintained under identical set of conditions. Observations on mortality were recorded after 24 h of fumigation.

In treatment B, where irradiation was preceded by fumigation, insects were first fumigated with various fumigants, (close to LC_{50} doses) and the survivors after 24 h and 7 days were subsequently exposed to gamma radiation doses of 2-12 krad in case of larvae and 8-18 krad for adults. Each treatment was replicated three times with 25 insects in each replication. Two sets of control–one with irradiated insects and another with untreated (normal) insects were maintained for this group of treatment also. Observations on the mortality were recorded at 3 day interval up to

15th day. Mortality data, 12 days after irradiation, were considered for calculating LD_{50} values.

Mortality data for both the treatments were subjected to probit analysis (Finney 1971).

RESULTS AND DISCUSSION

The LC_{50} value for fumigation of 10 day old larvae was determined to be 18 mg/L for EDCT, 6.9 mg/L for EDB, 2.5 mg/L for CS_2, and 0.8 mg/L for MB; whereas for 8-10 day old adults, the LC_{50} value for fumigation was 42 mg/L for EDCT, 9.2 mg/L for EDB, 7.5 mg/L for CS_2, and 1.2 mg/L for MB. The LD_{50} values for irradiation was determined to be 5 krad for 10 day old larvae and 10 krad for 8-10 day old adults of *T. castaneum*. Further, the toxicity of EDCT, EDB, CS_2 and MB to the larvae and adults of *T. castaneum* before and after exposure to gamma radiation was determined.

Data on the toxicity of EDCT, EDB, CS_2 and MB to the larvae and adults of *T. castaneum* after irradiation indicated that exposure to gamma treatment of larvae and adults followed by fumigation either within 6 h or after 7 days, affected their susceptibility to the fumigants. Larvae and adults exposed to doses of 5 and 10 krad, respectively when fumigated within 6 h required higher doses of the fumigants at LC_{50} level. However, if the fumigation was done 7 days after irradiation, comparatively lower doses of the fumigants were needed to achieve similar results. For instance, for irradiated larvae, the LC_{50} values for EDCT were 23.63 and 15.54 mg/L when fumigated within 6 h and after 7 days, respectively, as against 21.73 mg/L for control. Similarly, the LC_{50} values of EDCT for irradiated adults were found to be 48.75 and 37.59 mg/L when fumigated within 6 h and after 7 days, respectively, as compared to 40.12 mg/l for control.

In the next phase, the effect of different doses of gamma radiation on the larvae and adults after fumigation at LC_{50} doses was evaluated. The results did not reveal any significant difference in the susceptibility of fumigated larvae or adults when the survivors of the fumigation treatment were irradiated either within 24 h or after 7 days. When the larvae were fumigated with EDCT at a dose of 18 mg/L and then subjected to different doses of gamma radiation, then LD_{50} values were found to be 4.07 and 4.09 krad at 24 h and 7 days after fumigation, respectively, against 4.05 krad for control. Similarly, when adults were first fumigated with EDCT at a dose of 42 mg/L (LC_{50} value) and exposed to gamma radiation thereafter, the LC_{50} values were 10.56 and 10.40 krad for 24 h and 7 days after fumigation treatments respectively, as compared to control (10.31 krad). Identical trends in both the treatments were observed in case of other fumigants, tested (Tables 1 and 2). The comparison of LD_{50} doses of gamma radiation for fumigated and non-fumigated larvae and adults showed that these values did not differ statistically. This suggests that fumigation of *T. castaneum* stages at their respective LC_{50} levels followed by irradiation of the survivors did not affect their susceptibility, as fumigated and non-fumigated insects remained almost equally susceptible to gamma radiation. It is thus evident that fumigation preceding irradiation did not predispose the insects to any change in their susceptibility level to gamma radiation.

TABLE 1

Interaction of fumigants and irradiation on susceptibility of *Tribolium castaneum* Larvae

Fumigant	LC_{50} (mg/L) value of fumigants for irradiated insects			LD_{50} (krad) value of gamma irradiation for fumigated insects		
	FG	**IR-FG (6h)**	**IR-FG (7days)**	**IR**	**FG-IR (24h)**	**FG-IR (7days)**
Ethylene dibromide-carbon tetrachloride	21.72	23.62	15.53	4.05	4.07	4.09
Ethylene dibromide	7.26	8.83	6.17	4.05	4.20	4.49
Carbon disulphide	2.98	4.48	2.82	4.05	4.73	4.83
Methyl bromide	0.95	1.15	0.78	4.05	4.41	4.20

IR: Irradiation; FG: Fumigation; IR-FG: irradiation followed by fumigation;

FG-IR: Fumigation followed by irradiation

The use of varied short radiation doses of about 0.2-0.5 kGy provides another alternative in the control of pests in store. Combined treatments of radiation and carbon dioxide produced a higher mortality in *T. confusum* than did either treatment alone (Omsar *et al.*, 1988). This method has the advantage of leaving no residues in the product, though it might not be feasible due to the high costs involved in application. Other experiments involved the use of microwave energy against stored product pests. A special microwave unit was developed with a variable speed conveyor belt and tested for insect control in stored milled rice. Results indicated that *Tribolium confusum* and *Cryptolestes pusillus* can be killed economically with microwave energy (Langlinais, 1989). Pests and life stages vary in sensitivity to radiation. Irradiation is most accurately called a pest control measure, because the process can and does kill most pests at the doses commonly used. The stored product pests (or life stages) that are not immediately killed are sterilized and/or prevented from further growth and development.

Cautious perusal of literature on the interaction of gamma radiation and fumigation revealed that this aspect has not received adequate attention. Cogburn and

Gillenwater (1972) had some similar observations and they showed that in *T. confusum,* low doses (2-6 krad) usually reduced the effectiveness of methyl bromide when fumigation was accomplished within 24 h after irradiation. Higher radiation doses (10-50 krad) followed 7 days later by methyl bromide fumigation generally killed more insects than did either treatment when applied alone. Delay of 7 days between irradiation and fumigation with methyl bromide increased insect mortality but reversing the sequence, *i.e.,* when the survivors of methyl bromide fumigation were subjected to irradiation, there was no increase in mortality showing no interaction, between the two treatments. Results obtained in the present study are in consonance with the findings of Cogburn and Gillenwater (1972). In the case of phosphine fumigation also, these workers did not observe any interaction between the fumigant and gamma radiation in *T. confusum.*. Similar results were reported by Saxena and Bhatia (1981) in *T. castaneum* where they did not find any interaction between the two treatments whether irradiation preceded or followed fumigation with phosphine. Results obtained in the present study with other fumigants (EDCT, EDB and CS_2) are in conformity with the findings discussed above.

TABLE 2

Interaction of fumigants and irradiation on susceptibility of *Tribolium castaneum* adults

Fumigant	LC_{50} (mg/L) value of fumigants for irradiated insects			LD_{50} (krad) value of gamma irradiation for fumigated insects		
	FG	**IR-FG (6h)**	**IR-FG (7days)**	**IR**	**FG-IR (24h)**	**FG-IR (7days)**
Ethylene dibromide-carbon tetrachloride	40.12	48.75	37.58	10.30	10.56	10.40
Ethylene dibromide	10.49	12.11	8.90	10.31	10.23	10.50
Carbon disulphide	7.19	9.52	6.49	10.31	10.91	10.12
Methyl bromide	1.33	1.56	1.19	10.31	10.75	10.36

IR: Irradiation; FG: Fumigation; IR-FG: irradiation followed by fumigation;

FG-IR: Fumigation followed by irradiation

Our findings further suggest that irradiation of *T. castaneum* larvae and adults immediately before fumigation may protect insects. The reason for this is not clearly understood. However, it is on record that sublethal doses of radiations often cause a reduction in the metabolic rate of affected animals. This could have lowered respiration rate of the insects resulting in reduced absorption of the fumigants (Cogburn and Gillenwater, 1972). This seems to be partly true, because higher range of sublethal gamma doses if reacted in the similar manner would have further increased the resistance of insects towards fumigants but that was not the factual situation. This could be explained by another hypothesis, that is, low doses may cause 'radiation hormesis' by stimulating the immune system. On the other hand, if the irradiated insects were fumigated after a gap of 7 days the insects showed higher degree of susceptibility to the fumigants. This indicated that irradiation might have altered the immune responses and modulated the susceptibility towards fumigants.

The irradiation of bagged grains or cereals is practical, effective and immediately available in many countries. The only barriers are the lack of regulatory approval in some countries and the lack of irradiation equipment. Grains are often handled in bags in developing countries under food security programs. This technology is available now and should be more used and promoted as a pest control method, particularly where use of methyl bromide as disinfectant is being phased out due to its ozone-depletion effect, or where phosphine pest resistance is a concern, or a reality. There are outstanding questions about the practicality of irradiating bulk grains and cereals. At present, the lack of information and resolution of these issues may deter investment in irradiation for bulk grain.

However our study suggests that by integrating the radiation technology, the effectiveness of management modality using fumigants could be enhanced towards the insect-pests that have developed resistance against currently used fumigants. Also in future, a novel strategy might be devised by coupling irradiation with fumigation / biorational measures for effective management of stored product pests.

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