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Irradiation as a Potential Phytosanitary Treatment for the Mango Pulp Weevil *Sternochetus frigidus* (Fabr.) (Coleoptera: Curculionidae) in Philippine Super Mango

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Irradiation was explored as a method of quarantine disinfestation treatment for the mango pulp weevil *Sternochetus frigidus* (Fabr.). *S. frigidus* is an important quarantine pest preventing the export of mangoes from the Philippines to countries with strict quarantine regulations. Mangoes obtained from Guimaras Island are exempt from this ban as they are certified to be free from seed weevil and pulp weevil infestation.

In the dose-response tests, *S. frigidus* larvae, pupae and adults in mangoes were irradiated at target doses of 25, 50, 75, 100, 150, 300 and 400 Gy. The number of eggs laid by adult females decreased with increasing dose. Treatment with irradiation doses of $\geq$75 Gy resulted in sterility in adults developing from larvae and pupae while doses of $\geq$100 Gy resulted in sterility in irradiated adults. The adult was the most tolerant stage based on sterility or prevention of adult reproduction. Significant differences were observed in adult longevity among treatment doses in *S. frigidus*, but none between sexes and in the interaction between dose and sex.

Key Words: disinfestations, mango pulp weevil, postharvest phytosanitary treatment, quarantine pest, *Sternochetus frigidus*

Abbreviations: AQIS – Australian Quarantine Inspection Service, DUR – dose uniformity ratio, MPW – mango pulp weevil, PNRI – Philippine Nuclear Research Institute, USDA-APHIS – United States Department of Agriculture - Animal and Plant Health Inspection Service

INTRODUCTION

The presence of the mango pulp weevil (MPW) *Sternochetus frigidus* (Fabr.), a quarantined pest in Palawan, has prevented the export of mangoes from the Philippines to the U.S. and other countries with strict quarantine regulations. The export ban, however, does not include mangoes obtained from Guimaras Island which have been certified as seed weevil-free and pulp weevil-free by the United States Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS) and the Australian Quarantine Inspection Service (AQIS). The area planted to mango in Palawan is 1,872 ha where ‘Carabao’ mango, the commercial variety, covered 1,400 ha or about 75% of hectarage. Production of mango in 2011 was 7,020.42 mt where ‘Carabao’ mango consisted of 4,782.43 mt or about 68% (BAS 2011). Other varieties of mangoes found in Palawan are ‘Mampalang’, ‘Wani’, ‘Katchamita’, ‘Pico’ and apple mango (De Jesus and Gabo 2000). The local ‘Carabao’ mango is known internationally as Philippine super mango.

As early as 1987, numerous surveys have been done to confirm the presence of mango seed weevil that was erroneously reported to be present in the Philippines. To date no mango seed weevil has been found but a closely related species, the mango pulp weevil (MPW) *S. frigidus,* was reported in 1987 in the southernmost towns of Bataraza and Brooke’s Point in Palawan province (Basio et al. 1994). *S. frigidus* (Fabr.) (syn. *S. gravis)* (Coleoptera: Curculionidae) is a major pest of mango (CPC 2001), and is known to occur in Northeastern India, Bangladesh, Indonesia, Thailand, Myanmar, Malaysia, Singapore and the Philippines. MPW infestation in the Philippines has been confined to central and southern Palawan while the northern part of Palawan is still uninfested (De Jesus and Gabo 2000).

The life history, host range, feeding and reproductive behavior of MPW were studied by De Jesus and Gabo (2000), De Jesus et al. (2003 a, b) and De Jesus (2008). There is no visible external manifestation of MPW on fruit; the adult weevils leave the fruit through exit holes only when the fruit becomes rotten. Internal signs of infestation include brown tunnels in the flesh of mango and the presence of MPW larvae, pupae, or adults. The adults can live for 1½ yr and females may lay almost 800 eggs during their lifetime with a mean oviposition period of about 165 d (De Jesus 2008).

The difficulty of identifying infested fruit by visual inspection makes postharvest treatment necessary for disinfestation. Postharvest disinfestation of mango against mango weevils was not successful by using various treatments such as heat, cold and fumigation (Balock and Kozuma 1964). Balock and Kozuma (1964) thought that irradiation would work for the disinfestation of mango against mango weevils back then.

Irradiation is a viable alternative to disinfest mangoes of MPW. It does not leave toxic residues and does not make food harmful to human health (WHO 1994). The criteria used for the evaluation of efficacy of treatment is often based on reduction of product damage and prevention of population growth since immediate mortality in mature larvae, pupae or adults requires a very high dose, thereby affecting the quality of the product.

The mode of action of ionizing radiation as a quarantine treatment involves the breaking of chemical bonds that may prevent the normal development or reproduction of the organism; DNA molecules are the most likely to be affected (Hallman 2003). The physiological and biochemical effects of radiation in insects were reviewed by Nation and Burditt (1994). Gonads are sensitive to radiation; hence, insects surviving irradiation treatments should be sterile at recommended treatment doses (Tilton and Brower 1983).

The use of ionizing radiation as a quarantine treatment has been studied for a number of curculionid weevils, particularly *Conotrachelus nenuphar* (Herbst) (Hallman 2003), *Cylas formicarius elegantulus* (Follett 2006), and *Sternochetus mangifera* (Follett 2001).

No study on irradiation has ever been conducted on MPW. Previous irradiation research had focused on the mango seed weevil *S. mangiferae*, another important pest of mango that does not occur in the Philippines. Although small-scale research suggested that *S. mangiferae* might be sterilized at 100 Gy (Seo et al. 1974; Follett 2001), an irradiation dose of 300 Gy was eventually approved by the USDA-APHIS for quarantine control of this pest in imported mangoes based on low confidence in research supporting 100 Gy (USDA-APHIS 2002). The dose of 300 Gy set by USDA-APHIS has not been used commercially as this may be excessive for *S. mangiferae* (Hallman et al. 2010). Unlike in fruit flies, the development of quarantine treatment protocol against mango weevils has been difficult since the insect has only one generation per year and there is no artificial diet for rearing the insect in the laboratory. Therefore, the use of Probit 9 (99.9968% mortality) level testing is not practical for the mango weevils (Follett 2001).

In 2006, low-dose generic radiation treatments were approved for the first time. The USDA-APHIS approved generic doses of 150 Gy for tephritid fruit flies and 400 Gy for all insects except pupa and adult Lepidoptera (Follett 2009). The generic doses apply to all fresh agricultural commodities. The availability of generic radiation treatments has stimulated worldwide interest in phytosanitary uses of this technology. Hawaii uses the generic radiation treatments to export 5,000–6,000 tons of tropical fruits and vegetables to the United States mainland annually (Follett 2009). The 400 Gy generic dose is used to treat mangoes from India and Pakistan, several tropical fruits from Thailand, guava from Mexico and dragon fruit from Vietnam for export to the US (Hallman 2012).

Lowering dose levels below 400 Gy for treatment of MPW will be beneficial to Philippine super mango for it will minimize any fruit quality problems and increase the capacity of the treatment facility because of shorter treatment time.

This study was conducted to determine the most radiotolerant stage of *S. frigidus* and to identify a potential dose for an effective irradiation quarantine treatment to disinfest Philippine super mango of MPW.

MATERIALS AND METHODS

Source of Insects

The different stages of MPW test insects used in the irradiation studies were obtained from Brooke's Point, Palawan. Due to the absence of an artificial diet for MPW, mass-rearing of the insect was done under field condition in Palawan using developing mango fruits on mango trees as substrate. Pairs of male and gravid female MPW were introduced into bagged mango fruits at 65 d after flower induction, or approximately at the chicken-egg-size fruit stage. Dissection of fruit samples was done to ascertain weevil development (De Jesus and Gabo 2000). The infested mangoes with the desired stage of development (larva, pupa or adult) were securely packed in carton boxes and placed inside crates (5 boxes per crate) then shipped to Manila by plane and transported to the Philippine Nuclear Research Institute (PNRI) for irradiation studies. All shipments were made possible under a special quarantine permit issued by the Department of Agriculture - Bureau of Plant Industry and guarded by a Plant Quarantine Officer. Voucher specimens of adult MPW were deposited at the University of the Philippines Los Baños Museum of Natural History.

Irradiation

Irradiation was carried out at the PNRI Multipurpose Irradiation Facility. The facility has eight individually operated source racks and four turntables. The activity of the Co-60 source as of March 1, 2008 was 33.87 kilocuries.

The dosimetry system used in the irradiation of mangoes was prepared according to ASTM Standards (2004) and traceable to the National Physical Laboratory of the United Kingdom. Dose mapping was performed first on the mango boxes to determine the positions of the minimum and the maximum doses, the minimum dose rate and the dose uniformity ratio (DUR). During routine irradiation, dosimeters were placed outside the mango fruits but at the expected minimum and maximum positions for a four-sided irradiation, i.e., at the front (Plane I), middle (Plane II) and back planes (Plane III) to monitor the dose received by the mangoes (Fig. 1).

Dose-response Tests

The 5th instar larva, 2-d-old pupa, and the 2-wk-old adult MPW were irradiated using 25, 50, 75, 100, 150, or 300 Gy. An additional irradiation dose of 400 Gy for adults was conducted to estimate the dose required to prevent reproduction. An untreated lot served as the control. The experiment was conducted with three replications per treatment and 100 mangoes per treatment (dose) for all the tests. One hundred mangoes per trial were cut open at the time of irradiation to serve as check samples.

After conducting irradiation treatments, all test fruits (both treated and control mangoes) still in boxes were held in the laboratory for MPW development. Later, the fruits were cut open to remove the weevils. For test fruits with adult MPW, the schedule of cutting was 2 d after irradiation, or as soon as the mango fruits became partly ripened, for ease of removing the weevils. For larvae, the fruits were cut open 16 d after irradiation; and for pupae, 15 d after irradiation, to allow the surviving immatures to develop into adult weevils. The surviving adult weevils were held in perforated plastic containers and provided with fresh green mango fruit squares for continued development. About 10 d after survival, irradiated and untreated adults were separated by sex, following the procedure of De Jesus et al (2002).

Adult pairs of 10 males and 10 females were confined in perforated plastic containers for mating.

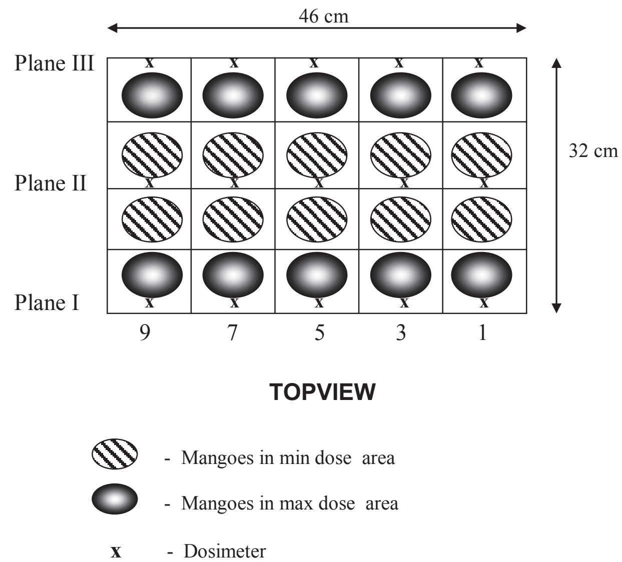

Fig. 1. Position of dosimeters and mangoes identified in the minimum and a maximum dose area inside the mango box (Drawing not to scale)

Mango fruit squares were provided and replaced daily together with the tissue paper lining for oviposition until the time when no more oviposition occurred. Eggs were collected by spraying the tissue paper with water and, by using a pair of fine-pointed forceps, the eggs were removed individually along with a small portion of the tissue paper to prevent damage to the eggs. The eggs were transferred onto a square black cloth provided with a wet absorbent cloth underneath in a covered petri-dish to prevent egg desiccation. Egg collections and mortality assessments were made daily.

The number of eggs laid per female and the percent egg hatchability were calculated. A similar procedure was done on the control weevils. The efficacy of treatments was estimated based on adult sterility or prevention of reproduction, i.e., no oviposition. The MPWs were held at a room temperature of 27.05 ± 0.93 °C, at 81.23 ± 7.14 % RH and a 12:12 (L:D) h photoperiod.

Statistical Analysis

To compare radiotolerance between life stages, data for estimates of the number of eggs per female were subjected to linear regression and analysis of covariance using the standard least squares model, with stage as a discrete effect and dose as a continuous effect (SAS Institute 2002). Data used in the regression model included 0 Gy (control), any radiation dose causing mortality between 0% and 100%, and the lowest dose causing 100% mortality. Residual plots were evaluated to ensure that regression model assumptions were met for each treatment combination. Covariance analysis requires the slopes of the regression lines fitted to each group to be parallel, therefore, the assumption of parallelism (non-significant life stage x dose interaction effect) was tested before evaluating intercepts (life stage effects) (Sokal and Rohlf 1981). Log transformed data were used in covariance analysis. To estimate the dose required to cause 100% adult sterility, untransformed data for estimates of the number of eggs per female for each life stage treatment were subjected to linear regression and the inverse prediction calculated. The longevity data (days to 100% mortality) were first tested for normality using Shapiro-Wilk and for homogeneity of variances using Levene's Test before proceeding to ANOVA using two-factor factorial in RCBD.

RESULTS AND DISCUSSION

Dose-response Tests

Shipments of MPW-infested mangoes from Palawan for the dose-response tests were made in May 2007 (adults) and from May to September 2008 (immatures). Percentage fruit infestation ranged from 43 to 57% infestation. Adult sex ratio (male: female) determined for each stage was 49.3:50.7, 51.7:48.3 and 50:50 for larva, pupa and adult-infested mangoes, respectively.

The effects of the different doses of irradiation on MPW larvae and pupae and on their development resulting in adult recovery and fecundity are shown in Tables 1 and 2. Adult recovery was lower in the larval than in the pupal stage. More than 60% abnormal adults (live and dead) (Fig. 2) developed from larvae irradiated with 25 and 50 Gy, and 32% from pupae irradiated with the same doses. This result indicates that the larval stage is more radiosensitive than the pupal stage. Due to the limited number of live adults developing from immatures (larvae and pupae) especially at higher doses, the number of males and females paired for the mating tests were lower in immatures than in adults. The fecundity of irradiated adult females developing from immature (larvae and pupae) was greatly reduced at doses of 25 and 50 Gy and completely inhibited at doses of $\geq$75 Gy. Egg hatchability in the control was 95.1% for larvae and 93.2% for pupae.

Table 3 shows the effect of irradiation of MPW adults in fruit on adult recovery and fecundity. Adult mortality was very low in treated adults. Unlike in immatures, the number of adults with morphological abnormalities recovered was very low (0–1%) in all doses used that may not be considered an effect of irradiation since abnormal adults were also observed from the untreated control. Similar to immatures, the fecundity of irradiated adult females (25–75 Gy) was also greatly reduced and completely inhibited at doses $\geq$ 100 Gy. However, eggs laid by females irradiated with 75 Gy did not hatch into larvae. The number of eggs laid per female per day decreased with dose (Fig. 3). Egg hatchability in the control was 89.3% (94,848 eggs), which is comparable to 90.3% (1,458 eggs) hatchability based only on 15 pairs of newly-emerged male and female adults (De Jesus 2008).

Table 4 shows the linear regressions on adult female reproduction based on estimated eggs/female at different developmental stages of MPW irradiated at doses between 0 and 150 Gy. Based on the predicted dose for 100% sterility at 95% confidence limit (CL), the order of decreasing radiosensitivity was larva > pupa > adult, which indicates that the adult stage was the most tolerant to gamma radiation. Our findings agree with those of other studies showing increased tolerance to radiation with increased level of development (Hallman et al. 2010).

Oviposition and percentage egg hatchability in the untreated control weevils was comparable with those of the weevils in the study of De Jesus (2008). This result suggests that the conditions at the PNRI holding laboratory were suitable for mating and/or oviposition by MPW.

The adult MPW is quite long-lived. For the longevity data, the assumption of normality was satisfied and when tested for homogeneity of variances, only four outlying points were observed; hence, we still proceeded with the parametric ANOVA analysis. Results showed that there were significant differences in adult longevity among treatment doses ($F_{7,\,14} = 45.34$, $p < 0.0001$) in *S. frigidus*, but none between sexes (male and female) ($F_{1,\,2} = 1.60$, $p = 0.2155$) and in the interaction between dose and sex ($F_{7,\,14} = 0.44$, $p = 0.8673$). Figure 4 shows the functional relationship of dose with adult longevity in *S. frigidus*. At 25 and 50 Gy, the life span of adult MPW was longer compared with the control. Hallman (2000) noted a similar phenomenon in a few other studies.

CONCLUSION AND RECOMMENDATIONS

The adult stage of the MPW, that is, the most developed stage, was the most tolerant to gamma radiation. This finding is important since a quarantine treatment must be effective against the most tolerant stage of the quarantine pest that may be shipped with the commodity. Because adult MPW may be present in packed mangoes, an irradiation treatment would have to ensure sterility or non-reproduction of the insect.

Based on the dose-response tests, a target dose of 100 Gy resulted in 100% sterility in adult MPW. This dose can be validated using a larger number of adult MPW that was found to be the most tolerant stage to gamma irradiation. Lowering the irradiation dose from 400 Gy (generic dose) to 100 Gy will not only lower the treatment cost but also minimize any deleterious effect on the quality of irradiated mango.

Table 1. Adult recovery and fecundity of irradiated mango pulp weevil (*Sternochetus frigidus*) larvae in mango fruit.

Target Dose (Gy)	Measured Dose (Gy)[a]	Reps[b]	Estimated Treated Samples	Adult Recovery					Fecundity			
				Live Normal	Live Ab-normal	Total Live	Dead Normal	Dead Ab-normal	No. Males	No. Females	Eggs Laid	Eggs per Female (±S.E)[c]
0 (control)	-	3	265	185	2	187	0	0	84	98	48,407	497.9 (31.3)
25	23.0–34.7	3	265	57	91	148	3	8	28	22	6,513	269.9 (69.1)
50	40.8–64.5	3	265	31	25	56	1	35	13	12	357	39.2 (38.1)
75	60.0–80.1	3	265	7	1	8	6	9	7	6	0	0.00
100	91.3–118.0	3	265	13	2	15	5	7	13	6	0	0.00
150	130.9–169.8	3	265	20	2	22	0	12	10	11	0	0.00
300	253.9–330.0	3	265	25	1	26	1	6	10	12	0	0.00

[a]Average value of minimum doses and average value of maximum doses.
[b] Replications, with 100 fruits per replicate.
[c]Calculated from 5 replicates of approximately 10 adult pairs.

Table 2. Adult recovery and fecundity of irradiated pupae of mango pulp weevil (*Sternochetus frigidus*) in mango fruit.

Target Dose (Gy)	Measured Dose (Gy)[a]	Reps[b]	Estimated Treated Pop[b]	Adult Recovery					Fecundity			
				Live Normal	Live Abnormal	Total Live	Dead Normal	Dead Abnormal	No. Males	No. Females	Eggs Laid	Eggs per Female (±S.E)[c]
0 (control)	-	3	189	409	3	412	1	2	129	124	49,978	378.0 (15.7)
25	16.6–34.0	3	189	235	100	335	4	13	119	113	32,223	276.6 (27.0)
50	36.3–60.7	3	189	222	33	255	3	41	106	105	6,191	59.2 (16.4)
75	56.0–84.2	3	189	164	28	192	0	25	86	76	0	0.00
100	91.4–109.4	3	189	159	15	174	0	26	68	80	0	0.00
150	135.0–162.1	3	189	174	12	186	1	16	79	91	0	0.00
300	267.3–321.8	3	189	186	21	207	2	8	75	78	0	0.00

[a]Average value of minimum doses and average value of maximum doses
[b] Replications, with 100 fruits per replicate.
[c]Calculated from 5 replicates of approximately 10 adult pairs.

Table 3. Adult recovery and fecundity of irradiated adults of mango pulp weevil (*Sternochetus frigidus*) in mango fruit.

Target Dose (Gy)	Measured Dose (Gy)[a]	Reps[b]	Adult Recovery					Fecundity			
			Live Normal	Live Abnormal	Total Live	Dead Normal	Dead Abnormal	No. Males	No. Females	Eggs Laid	Eggs per Female (±S.E)[c]
0 (control)	-	3	479	3	482	7	0	150	150	94,848	632.3 (24.8)
25	21.6–35.3	3	431	0	431	9	0	150	148	64,766	431.8 (16.2)
50	44.0–58.0	3	449	0	449	11	0	150	150	6,924	46.2 (7.4)
75	68.7–84.9	3	447	1	448	5	0	148	150	30	0.2 (0.1)
100	93.4–114.4	3	515	1	516	4	0	150	150	0	0.00
150	139.9–168.1	3	456	0	456	3	0	150	150	0	0.00
300	276.5–322.6	3	394	1	395	7	0	150	150	0	0.00
400	379.6–440.4	3	622	4	626	17	0	150	150	0	0.00

[a]Average value of minimum doses and average value of maximum doses.
[b] Replications, with 100 fruits per replicate.
[c]To improve reproductive success, approximately 10 adult females were placed in plastic containers with an equal number of males.
To estimate the number of eggs laid per female, the total number of eggs per container was divided by 10. Standard errors were calculated from averages for five replicates of 10 females per treatment.

Table 4. Linear regression of adult female reproduction (estimated eggs/female) when mango pulp weevil at various life stages were irradiated at doses between 0 and 150 Gy.

Stage	Observations	Y-intercept	Slope	R^2	Predicted Dose (Gy) for 100% Sterility (No Egg Laying) (95% CL)
Larva	11	484.5 ± 30.9	−7.6 ± 0.9	0.80	64.0 (53.3–80.7)
Pupa	12	383.2 ± 16.8	–5.5 ± 0.4	0.83	69.7 (63.7–77.1)
Adult	12	561.3 ± 23.5	−6.8 ± 0.4	0.81	82.7 (77.7–88.3)

Regression analysis used data from Tables 1–3. Extrapolated values and confidence intervals for predicted dose for no oviposition were used to suggest a treatment dose to cause 100% sterility.

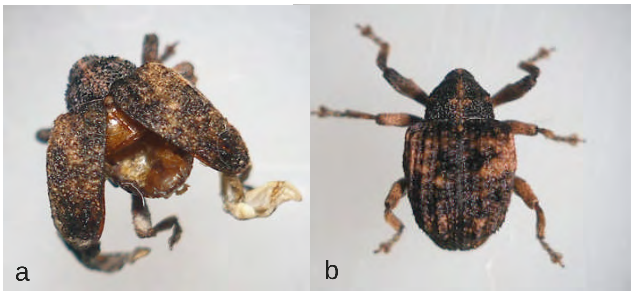

Fig. 2. Eclosed adult *Sternochetus frigidus* showing (a) morphological abnormalities and (b) normal (5.5 x 3.5 mm).

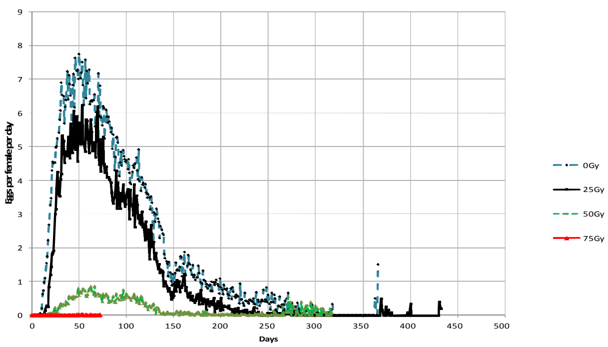

Fig. 3. No. of eggs laid per female per day by adult female *Sternochetus frigidus* at different doses of gamma radiation and in the untreated control. No oviposition occurred at ≥100 Gy (Adults irradiated).

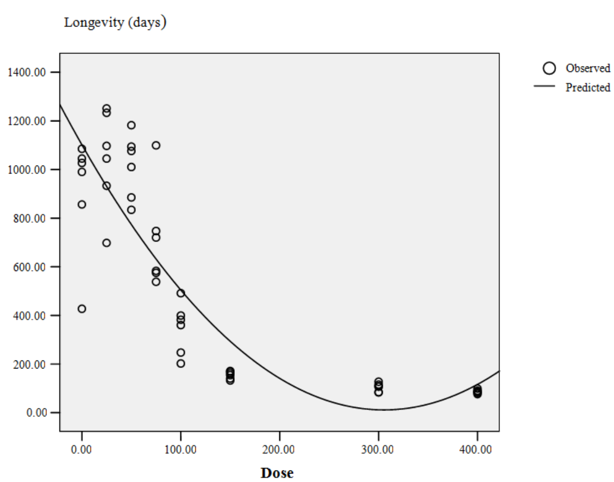

Fig. 4. Functional relationship of irradiation dose with longevity in adult *Sternochetus frigidus*.

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