GAMMA RADIATION: A SANITATING TREATMENT OF AFB-CONTAMINATED BEEKEEPING EQUIPMENT

GAMMA RADIATION SANITATION IN BEEKEEPING MANAGEMENT

Alessandra Baggio[1], Albino Gallina1, Nicoletta Dainese[1], Chiara Manzinello[1], Franco Mutinelli[1], Giorgia Serra[2], Roberto Colombo[2], Emanuele Carpana[2], Anna Gloria Sabatini[2], Klaus Wallner[3]; Roberto Piro[4], Emanuele Sangiorgi[4]

[1]National Reference Laboratory for Beekeeping;Istituto Zooprofilattico Sperimentale delle Venezie;Viale dell'Università, 10;35020 Legnaro (PD), Italy;Phone: +39 049 8084259;Fax: +39 049 8084258 ;e-mail: fmutinelli@izsvenezie.it
[2]Istituto Nazionale di Apicoltura,via di Saliceto, 80;40128 Bologna, Italy;
e-mail: agsabatini@inapicoltura.org
[3]Universität Hohenheim,Landesanstalt für Bienenkunde;70593 Stuttgart, Germany;
e-mail: bienewa@uni-hohenheim.de
[4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna,Via A. Bianchi, 9;25124 Brescia, Italy;e-mail: rpiro@bs.izs.it

ABSTRACT

Although American Foulbrood is well known, this disease is still a relevant cause of beehive and economic losses to beekeepers. Gamma radiation from a Cobalt-60 source provides an effective means of treating AFB contaminated beekeeping material for its reuse in apiary. Contaminated equipment (hive, frames, wax, honey) was irradiated at three different dosages: 10, 15 and 25 kGray. The infectivity of the spores contained in scales and honey was tested before and after the radiation treatment by culture. Furthermore, the gamma rays effects on beeswax and honey were investigated. None of the irradiated samples was positive to P. larvae irrespective of the radiation level applied. At a 10 kGray radiation level no particular changes in beeswax composition were registered. The main physico-chemical modifications observed in honey were those of enzymatic activity decrease, bubbles formation and leakage of honey out of frames.

Keywords: *American foulbrood, honey, gamma radiation, sanitation, wax*

INTRODUCTION

American foulbrood (AFB) is a serious disease of honeybee brood caused by spore-forming bacteria Paenibacillus larvae subsp. larvae. Although this disease is well known, AFB is still a relevant cause of beehive and economic losses to beekeepers. In fact, spores of P. larvae are very resistant and remain viable almost indefinitely in spore-contaminated hive equipment being a potential source of infection. Furthermore, AFB is characterized by an easy dissemination of spores within-colony and between colonies. Spores dissemination may occur naturally via swarming and robbing, but also artificially through certain apicultural practises such as use of

interchangeable combs and other hive parts, movement and sale of colonies. An efficient control of AFB can be obtained only when the detection of AFB-contaminated hives is rapid and their elimination is performed immediately. About the last crucial step, burning infected colonies is an effective way of killing spores, interrupting the disease cycle. However, since this results in hives destruction, it is not well accepted by beekeepers.

A very interesting method to decontaminate AFB infected equipment and to control AFB, is the use of gamma radiation(s). The first experimental studies were carried out in the ’70 which demonstrated the lethal effect of radiation treatments on AFB-spores without destructive action on equipment. However, if the biocidal action of gamma radiation(s) due to disruption of DNA is known, few information are available on the effects of gamma radiation on wax and honey. The aim of this work is to verify the sterilizing effectiveness of gamma radiation on AFB-contaminated apicultural equipment applied at different levels and the possible effects on physico-chemical characteristics of some beehive products as beeswax and honey.

Materials and methods

^{60}Co was used as source of gamma radiation. The samples were processed in duplicate and irradiated at three different levels: 10, 15 and 25 kGray. Gamma radiation treated equipment was analysed by four different laboratories according to matrix and parameters considered.

Hive equipment.

Three Dadant-blatt hives with AFB-contaminated combs were used. The infectivity of spores contained in scales was tested before and after the radiation treatment by culture.

Honey samples.

Three honey samples of different botanical origin were collected: Robinia, Honeydew and Multifloral. 28 samples of each honey (250 g/each) for physico-chemical analysis and 14 AFB-artificially contaminated samples for microbiological analysis were used, respectively. Honey samples were infected with 10^6 spores/g.

Physico-chemical analysis:colour (optical comparative method), moisture (refractometric method), pH, acidity (tritimetric method), glucose and fructose content (chromatographic method), hydroxymethilfurfural (HMF - chromatographic method), enzyme activities (diastase, α and β-glucosidase, glucoso-oxidase – colorimetric method), organic acids content (formic acid: enzymatic method; oxalic acid: chromatographic method) (Bogdanov et al., 1997) were considered.

Microbial analysis:AFB spores were detected by culture (MYPGP agar, 37°C for 4-5 days) using a positive control (DMS 7030). Further identification of P. larvae colonies was carried out under microscope after Gram stain and using biochemical analysis (catalase and nitrate test).

Beeswax samples.

14 beeswax samples (30 g/each) were tested for physico-chemical characteristics. Physico-chemical analysis: melting point, acid value, ester value, ratio number, free alcohols, free acids, hydrocarbons, esters (chromatographic method) were considered.

One-way and two-ways analysis of variance (ANOVA) were carried out to evaluate the effects of radiation treatment on physico-chemical parameters of beeswax and honey samples. When F-test value was significant, Newmann-Keul tests were used to detect differences between means. Linear regression model was applied to evaluate the relationship between parameters variation and radiation dose.

Results

Hive equipment

None of the irradiated samples was positive to P. larvae irrespective of the radiation level applied.

Honey samples

Physico-chemical analysis

The main physical modifications observed in honey were: bubbles formation, leakage of honey out of boxes, foamy scum on the surface, some cloudiness and light browning of Robinia honey samples (from 5 to 15 mmPfund). A decrease of viscosity in liquid honeys was observed, whereas crystallized honey treated at 25 kGray became liquid.

Concerning chemical parameters, the effect of radiation was significant ($F = 14.9$, $df = 36, 3.7$, $p < 0.001$). This effect was different in relation to type of honey and to type of parameters analysed. In particular, a statistically significant decrease of enzyme activity was registered with a complete destruction of -glucosidase in Robinia honey treated at 25 kGray (Table I). These changes were linearly correlated to the increase of radiation dose (Table II). Among the enzymes analysed, glucosoxidase resulted more “selective” than the others with a mean decrease of 20% in honeys treated at 10 kGray (Figure 1), whereas diastase activity was more sensitive because of its significant decrease (on average 15%) already after a radiation treatment at 10 kGray dose, particularly in the case of Robinia and honeydew (Figure 2). Formic and oxalic acids are some of the radiolytic products of carbohydrates. A mean increase of 26.2% in formic acid after 10 kGray irradiation was registered. This increase was not significant and within the range of natural content.

Table I. Enzyme activity (mean and standard deviation) of three different honeys treated at three different radiation doses (10, 15 and 25 kGray). In each column same letters marks no-significant difference (Newmann-Keul test).

treatment dose (kGray)	Robinia	Multifloral	Honeydew	Robinia	Multifloral	Honeydew
	Diastase (Schade unit)			glucosoxidase (μg H_2O_2/g x h)		
0	9.55 ± 0.21a	29.75 ± 0.64a	44.90 ± 0.14a	49.30 ± 7.21a	76.20 ± 9.19a	43.20 ± 6.93a
10	7.35 ± 0.49b	28.20 ± 0.42b	36.55 ± 0.42b	28.10 ± 0.42a	75.45 ± 5.73a	35.00 ± 1.84a
15	7.33 ± 0.04b	27.78 ± 0.46b	35.38 ± 0.18c	16.65 ± 3.18a	61.05 ± 0.64a	33.25 ± 1.06a
25	5.88 ± 0.04c	22.58 ± 0.18c	29.88 ± 0.11d	9.15 ± 1.48b	55.30 ± 7.21b	27.75 ± 6.86a

treatment dose (kGray)	Robinia	Multifloral	Honeydew	Robinia	Multifloral	Honeydew
	α-glucosidase (mg pNPG/min x kg)			β-glucosoxidase (mg pNPG/min x kg)		
0	4.33 ± 0.78a	16.33 ± 0.08a	21.38 ± 2.07a	3.03 ± 0.02a	7.09 ± 0.95a	7.40 ± 0.62a
10	4.24 ± 0.22a	13.61 ± 1.08a	16.55 ± 1.89a	2.97 ± 0.04a	7.41 ± 0.22a	6.90 ± 0.26a
15	1.40 ± 0.38a	12.54 ± 3.11a	19.98 ± 0.17a	1.21 ± 0.12b	4.96 ± 0.18b	4.44 ± 0.45b
25	0.00 ± 0.00b	9.36 ± 1.34b	15.12 ± 0.71b	1.66 ± 0.21b	4.42 ± 0.23b	4.08 ± 0.04a

Table II. Values of linear relation between each enzyme activity analysed and radiation treatment dose.

Activity enzyme	b	a	R^2
Diastase	- 0.37	27.54	0.95
a-glucosidase	- 0.22	13.98	0.96
b-glucosidase	- 0.1	6.02	0.74
glucosoxidase	- 1.23	56.73	0.99

Figure 1. Glucosoxidase determination before and after radiation treatment at 10, 15 and 25 kGray of three different honeys (*Robinia*, Honeydew, Multifloral).

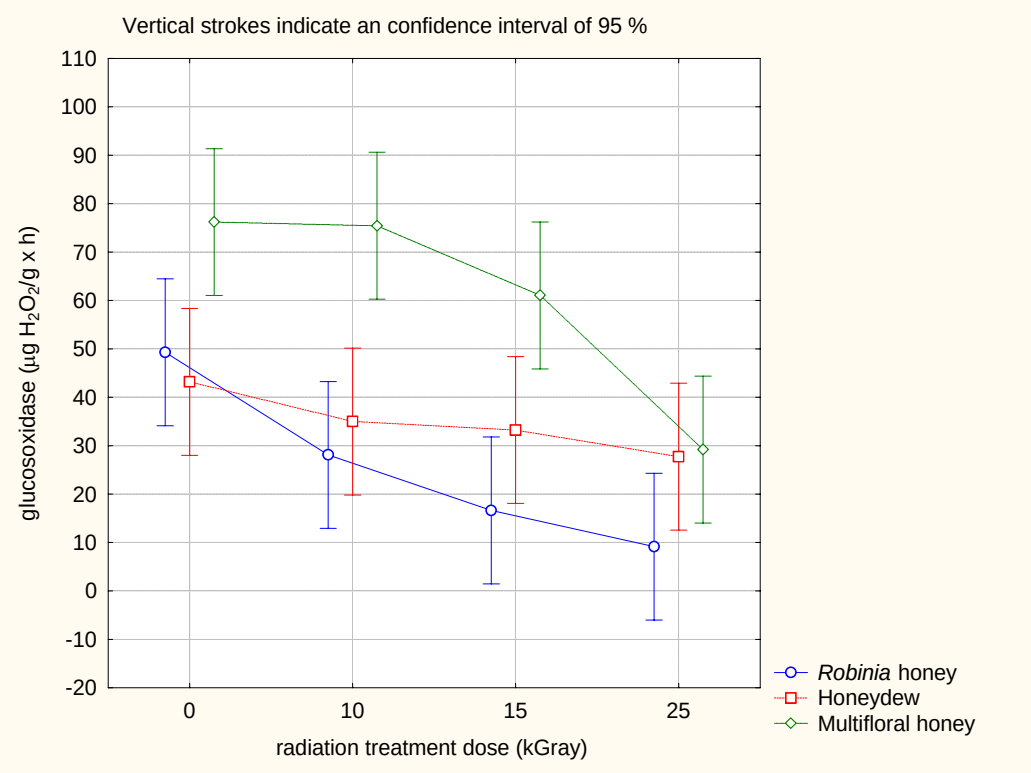
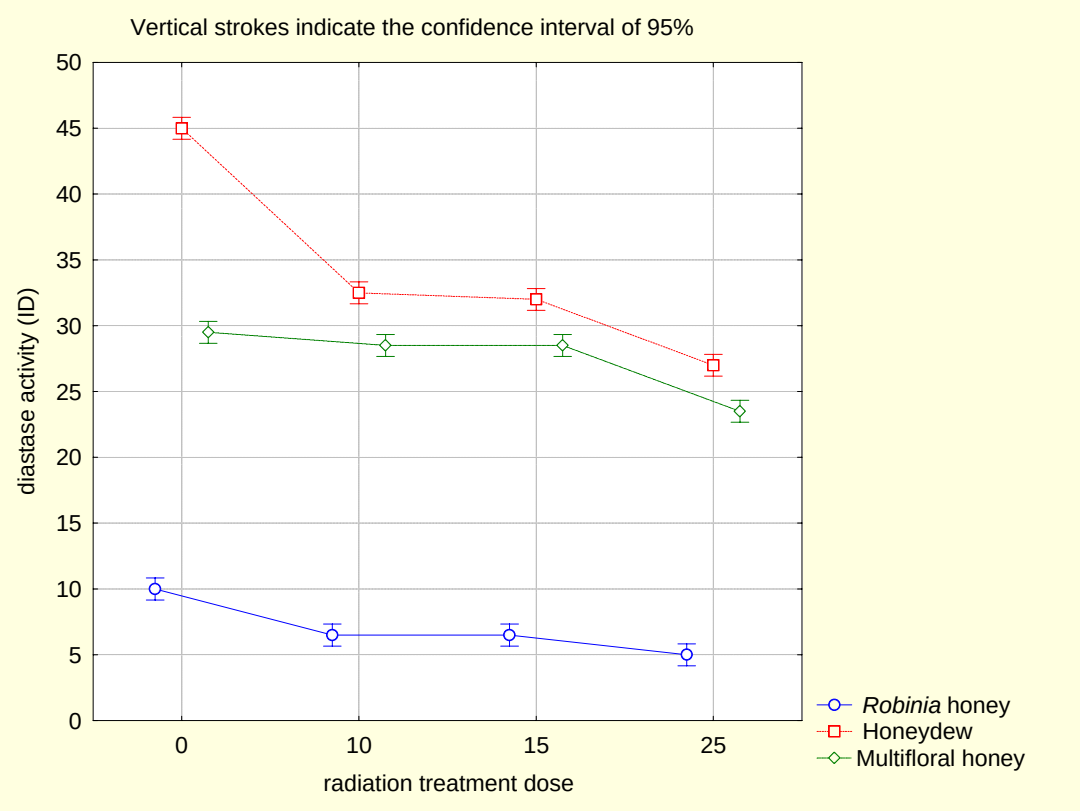


Figure 2. Diastase activity determination before and after radiation treatment at 10, 15 and 25 kGray of three different honeys (*Robinia*, Honeydew, Multifloral).



Microbial analysis

After irradiation at all levels AFB-spores lost their infectivity. In fact, all the samples analysed were negative on culture.

Beeswax samples

Physico-chemical analysis

In general, the effect of gamma radiation on beeswax samples was not significant (F = 1.0, df = 12, 2.9, p = ns). A slight increase of melting point resulted proportional to radiation level, but this difference was not significant.

Discussion

According to the literature (Gochnauer and Hamilton, 1970; Hornitzky and Willis, 1983), a 10 kGray dose radiation treatment provides the inhibition of AFB-spore germination in scales and in honey. Furthermore, at this level no particular changes in beeswax composition were observed. According to Katznelson and Robb (1962) and Wooton et al. (1985), the main modifications observed in honey were of physical changes. Among chemical properties, enzyme activity decreased with the increase of radiation level and, diastase activity was reduced already after a 10 kGray radiation treatment. HMF content before and after radiation treatment did not change as demonstrated by Wooton et al. (1985), according also to its low content in the investigated samples.

In conclusion, gamma radiation treatment at 10 kGray could really be of additional value to beekeepers as routine sanitating treatment of beekeeping equipment. A large scale application of this treatment is then recommended.

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