Vol. 159,
2022

Working Group „Integrated Protection of Stored Products“

Preceedings of the 13th Meeting
at Barcelona (Spain)

03-06 October, 2022

Edited by:

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IOBC-WPRS

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The content of the contributions is in the responsibility of the authors.

The IOBC-WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC-WPRS).

Le Bulletin OILB-SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Regionale Ouest Paléarctique (OILB-SROP).

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ISBN 978-92-9067-345-3

Web: http://www.iobc-wprs.org

Darmstadt, 2022

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Biorational CO_2 fumigation of sunflower and common bean: insecticidal potential and effect on seed vitality and quality

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Abstract: Store product pests often cause high qualitative and quantitative losses to seeds of agricultural plants during storage. Damages inflicted to a high category seed result in reduced germination that practically affects agricultural production. Therefore, it is important to control insect pests and mitigate losses in storages, but at the same time to preserve the germination potential of the seeds as well as their vitality and quality. Fumigation with CO_2 is a biorational method used for controlling store product pests in a variety of commodities. Its insecticidal potential is well documented, however the information about the effect on seeds is lacking. In this work, we assessed the efficacy of CO_2 fumigation and its effect on vitality (germination energy and germination) and quality (fatty acid composition) of sunflower and common bean seeds. CO_2, (62, 93 and 96 %), was applied to sunflower seeds artificially infested with *Plodia interpunctella* larvae and common bean infested with *Acanthoscelides obtectus* adults, in gas-tight bags. The lowest concentration (62 %) caused total mortality (100 %) of *P. interpunctella* larvae after 7 days of exposure. The two highest CO_2 concentrations caused relatively high mortality after two hours of exposure (81 and 86 %), while total mortality (100 %) was achieved after 24 h. The lowest concentration caused only 62.0 % mortality of *A. obtectus* after 24 h of exposure. In treatments with 93 and 96 % of CO_2 mortality was 88 and 93 % after 24 h exposure, respectively. Fumigation with CO_2, irrespective of concentrations, showed no adverse effect on seed germination of sunflower (97.0 to 99.5 % in all treatments) or common bean (91.3-95.3 %), or on the percentage of detectable fatty acids in sunflower seeds. However, varietal differences should be considered.

Key words: Carbon dioxide, oil crops, protein crops, controlled atmosphere, germination

Introduction

Controlled atmosphere (CA) treatments for suppression of stored product pests have received increasing scientific attention during the last 25 years. In those treatments, the atmospheric composition is controlled and maintained at a level lethal to insects or microbes (Adler et al., 2000). Carbon dioxide (CO_2) with low residual O_2 levels is mainly used to control pest organisms and to prevent stored product quality reduction. CO_2 fumigation represents a very effective biorational alternative to chemical fumigants. It is widely used for a variety of commodities, and the efficacy against insect pests is well documented. However, the information on its effect on seed vitality is scarce. This information is important especially for high category of oilseeds because, compared to legumes or cereal grains, these seeds have high content of oil and fatty acids which makes them more susceptible to deterioration.

This work aimed to assess the fumigation efficacy of CO_2 on *Plodia interpunctella* larvae and *Acanthoscelides obtectus* adults and its effect on the vitality of sunflower and common bean seeds (germination energy and germination) as major oil and protein crops.

Materials and methods

CO_2 was applied at different concentrations (62, 93 and 96 %) to sunflower seeds (oily variety Oliva) artificially infested with *P. interpunctella* larvae and to common bean seeds (variety 20tica) infested with *A. obtectus* adults, in gas-tight bags. The mortality of *P. interpunctella* larvae was observed after four different exposure periods (1, 2 and 24 h, and 7 days), while due to the short life of *A. obtectus* adults, 7 to 14 days (Golob et al., 2002), the mortality was recorded after 1, 2 and 24 h. All mentioned exposures represented different subtreatments. The experiment was carried out under controlled conditions at 27 ± 2 °C. The level of CO_2 in bags was measured after seven days, to confirm satisfactory gas-tightness of the bags used. Germination energy – GE and germination – G were assessed after four and ten days for sunflower and five and nine days for common bean. For sunflower seeds, fatty acids composition was determined as well.

Data were analyzed using one-way ANOVA and Duncan's multiple range test in statistical software SPSS 17.

Results and discussion

The lowest CO_2 concentration (62 %) was effective in suppressing *P. interpunctella* larvae but only after 7 days of exposure, when total mortality (100%) was recorded. Two highest CO_2 concentrations caused relatively high mortality after two hours of exposure (81 and 86 %), although larval recovery of 22 % occurred rapidly after the bags were opened. At these concentrations, the total mortality (100 %) without any larval recovery was achieved after 24 h. The lowest concentration (62 %) managed to cause 60.5 % mortality of *A. obtectus* but only after 24 h. Two highest CO_2 concentrations (93 and 96 %), however, caused relatively high mortality of this pest that was 88.0 and 93.6 % after 24 h exposure, respectively. The exposure to 93 % CO_2 after 1 and 2 hours enabled the recovery of almost 36.1 and 37.3 % of specimens, respectively, and at 96.0 % of CO_2, 14.5 and 9.7 %, respectively. The results are presented in Figure 1. Ofuya and Reichmouth (1992) reported similar results. Namely, at 88 % of CO_2, it took 5 days to achieve total mortality of *A. obtectus*. According to Bera et al. (2004), the complete control of the majority of insect populations was achieved with 20 % concentration of CO_2 within two months of storage period.

Controlled atmosphere, i. e., conditions of low O_2 and elevated CO_2 have been used for many years to control insect pests (Bell, 2000; Jayas and Jeyamkondan, 2002) and can be considered as efficient alternatives to toxic chemical fumigants for protection of stored seeds. This was also proven in our work, given the high mortality of both tested insect pests at the chosen levels of CO_2.

Fumigation with CO_2, irrespective of concentrations, showed no adverse effect on sunflower seed germination that ranged from 97.0 to 99.5 % in all treatments, while the control ranged from 93.0 to 98.0 %. GE and G of common bean, regardless of the variety, were not significantly affected by the different levels of CO_2 applied, although both parameters were slightly higher in all treatments (68.7-74.0 %, 91.3-95.3 %, respectively) compared to the control (59.3 and 84.7 %, respectively), as presented in Table 1. However, the possible varietal differences must be considered, thus it is recommended to perform preliminary fumigation of seed samples prior to large scale fumigation. Also, it is important to mention that the total germination (G) achieved in all treatments was higher than the minimal germination stipulated by the national Regulation on the quality of agricultural seeds (Off. Gazzettee, 34/2013-67), which is 85 % for sunflower and 70 % for the common bean. This is in accordance with the results of the Bera et al. (2004) who mentioned that the storage in CO_2-rich atmospheres, irrespective of concentrations and exposure periods, showed no adverse effect on germination and vigor of wheat seeds. Other authors suggested that altered atmospheric composition, primarily high levels of CO_2, preserved grain quality and maintained high level of germination in the stored grain (Banks, 1981; Bera et al., 2008). Results are also in accordance with Gvozdenac et al. (2021) who reported that high levels of CO_2 (over 60 %) had no adverse effect on germination energy and germination of oil-seed rape seeds.

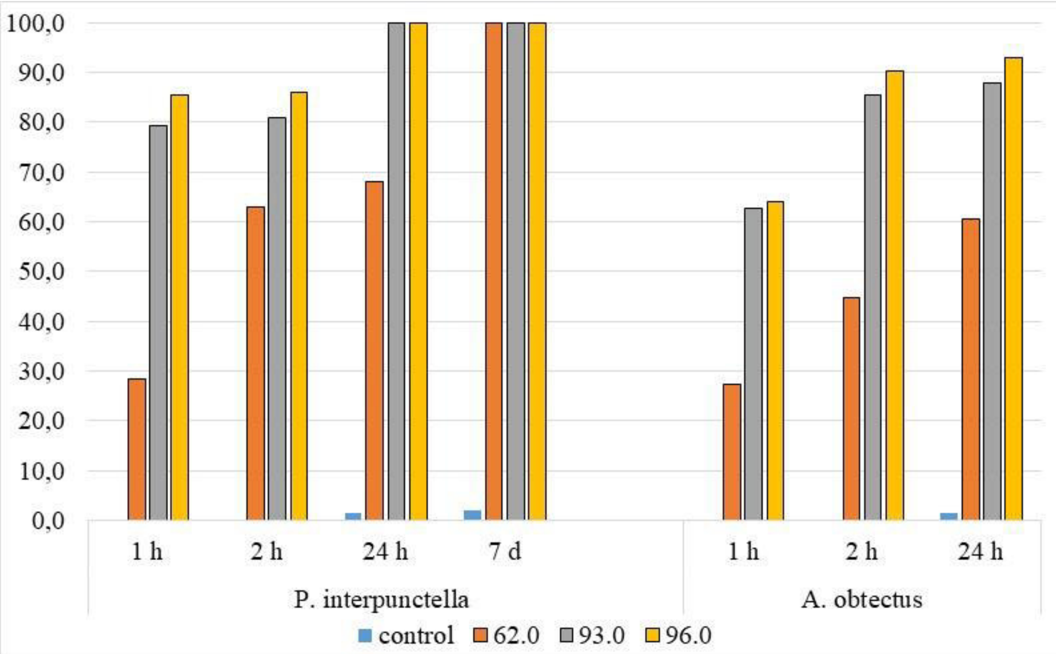

Figure 1. Mortality (%) of *P. interpunctella* larvae and *A. obtectus* adults in the different CO_2 treatments applied.

Table 1. GE and G of sunflower and common bean depending on the CO_2 concentration applied.

CO_2 %	sunflower		common bean	
	GE	G	GE	G
control	93.0 ± 1.0 b	98.0 ± 1.0 a	59.3 ± 2.3 c	84.7 ± 1.3 b
62	97.5 ± 0.5 a	98.0 ± 2.0 a	68.7 ± 1.3 b	94.7 ± 0.3 a
92	97.0 ± 0.0 a	97.0 ± 1.0 a	70.0 ± 1.0 ab	95.3 ± 1.3 a
96	98.5 ±0 .5 a	99.0 ± 2.0 a	74.0 ± 2.0 a	91.5 ± 1.5 a
F value	7.44*	0.44NS	83.12*	45.07*

Mean values (± SD) with the same lowercase letters indicate the same level of significance in rows-between CO_2 concentrations ($\alpha = 0.05$); NS P > 0.05, *P < 0.05; ** P < 0.01.

In our work, GE and G of common bean were significantly inhibited in the control, in comparison to CO_2 treatments. We can speculate that this is due to fungicidal or fungistatic effect of CO_2. Gupta et al. (2014) reported that CO_2 at 60-80 % concentrations reduced the incidence of several storage fungi (*Curvularia lunata*, *Cladosporium* sp., *Rhizopus stolonifer* and *Alternaria alternata*) on stored paddy seed. But 80 % CO_2 was required to control *Aspergillus flavus*.

Table 2. Content of fatty acids depending on the CO_2 treatment.

Fatty acids	**CO_2 treatment (%)**			
	control	**62**	**92**	**96**
Butyric C4:0	0.012	0.008	0.014	0.006
Caproic C 6:0	0.000	0.000	0.000	0.000
Capric C 10:0	0.034	0.025	0.031	0.003
Lauric C 12:0	0.775	0.726	0.766	0.026
Tridecanoic C 13:0	0.011	0.006	0.009	0.000
Myristric C 14:0	0.044	0.035	0.037	0.033
Pentadecanoic C 15:0	0.020	0.032	0.017	0.016
Pentadecanoic C 15:1	0.012	0.012	0.011	0.008
Palmitic C 16:0	4.45	4.03	4.09	4.18
Palmitoleic C 16:1	0.115	0.086	0.092	0.092
Heptadecanoic C 17:0	0.063	0.053	0.058	0.056
cis-10-HeptadecanoicC 17:1	0.175	0.341	1.274	0.154
Stearic C 18:0	3.52	3.70	3.81	4.18
Oleic C 18:1c+t	70.92	78.98	75.94	80.75
Linoleic C 18:2 c+t	14.51	6.21	9.45	6.98
gamma Linolenic C 18:3 n-6	0.796	0.588	0.418	0.151
Linolenic C 18:3n-3	0.062	0.121	0.122	0.044
Arachidic C 20:0	0.304	0.326	0.330	0.412
Eicosanoic C 20:1	0.196	0.208	0.210	0.253
Eicosadienoic C 20:2	0.008	0.000	0.000	0.011
C 20:3n6+C 21:0	0.009	0.010	0.009	0.011
C 20:5	0.153	0.110	0.104	0.037
Behenic C 22:0	0.943	1.030	0.992	1.315
C 22:2	0.169	0.101	0.139	0.080
C 23:0	0.000	0.000	0.000	0.014
Eruca C 22:1	0.042	0.064	0.063	0.071
Lignoceric C 24:0	0.327	0.379	0.339	0.492
C 22:6+C 24:1	0.000	0.024	0.025	0.018
total % of detected acids	**97.67**	**97.20**	**98.34**	**99.40**

The chemical analysis of the sunflower seeds indicates no statistically significant differences in the total percentage of detectable oil or in the composition of fatty acids (Table 2). However, there were some differences in the specific fatty acid content depending on the CO_2 treatment.

Acknowledgements

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number: 451-03-68/2022-14/200032. This work was done as a part of activities of the Centre of Excellence for Innovations in Breeding of Climate Resilient Crops – Climate Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia.

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