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Short Communication

Exposure to airborne endotoxin in Italian greenhouses: environmental analyses

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Abstract: The peculiar characteristics of the greenhouses as confined spaces, microclimate and poor air exchange with the outside environment, encourage the development of a large number of biological agents. Endotoxin, is probably a major causative agent of occupational health problems. The objective of this study was to measure the concentrations of airborne endotoxin in greenhouses with different cultures. The influence of microclimate was studied in correlation with endotoxin levels and type of cultured vegetables. The data indicate that workers employed greenhouses are exposed to low levels of inhalable endotoxins; endotoxin concentrations do not correlate with the temperature and relative humidity values. A strong correlation between the leaf size and endotoxin concentration was observed. The mean concentration of endotoxins in the air of greenhouses is relatively low, however, there could be peaks of exposure during harvesting and eradication of broadleaf plants.

Key words: Endotoxin, Greenhouse, Microclimate, Leaf, Occupational Exposure

In Europe, among the countries with the largest greenhouse areas, Italy is in the third place (26,500 ha), after Spain and Turkey (source: Eurostat 2007). In particular, agriculture is the main occupation in southern Italy. It is well documented that agricultural workers are exposed to high concentrations of bioaerosol due to the handling of large amounts of fresh and dried plant materials[1)], but less is known about exposures in greenhouses, where the confined spaces could generate different risk factors whose synergies may be harmful to human health. The peculiar conditions in these environments, as the microclimate and the poor air exchange with the outside environment, encourage the presence of organic dust and the consequent development of a large number of biological agents (molds, bacteria, pollen, endotoxin and mycotoxin)[2)]. A further source of exposure to microorganisms may be the use of organic fertilizers and of microbial pest control agents (MPCAs), which are beneficial microorganisms applied in crop production[3−5)].

In particular, endotoxin, an outer membrane component of Gram-negative bacteria, is ubiquitous contaminant of organic dust and is probably a major causative agent in health problems associated with organic dust exposure[6)]. Many occupational studies have shown positive associations between their exposure and respiratory disorders, including asthma-like symptoms, chronic airway obstruction, byssinosis, bronchitis, and increased airway responsiveness[7)]. Unlike moulds, endotoxin has also been recognized as a causative factor in the etiology of occupational lung diseases, including non-allergic asthma and organic dust toxic syndromes[8)]. Non-allergic asthma reflect a non-immune-specific airway inflammation. In occupational medicine it has long since been recognized that a substantial proportion of work-related asthma symptoms

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Table 1. Description of the investigated environments and seasons

Name sample	Crop	Production environment	Number of samplers	Work tasks	Plant stage	Date of air sampling
J	Zucchini	Greenhouse	1	Harvesting	Mature	03 May 2010
K	Arugula	Greenhouse	1	Potting	Young	27 July 2010
A	Lettuce	Greenhouse	6	Potting	Young	23 October 2012
B	Chili Pepper	Greenhouse	6	Harvesting	Mature	06 November 2012
C	Zucchini	Greenhouse	6	Harvesting	Mature	20 November 2012
D	Chili Pepper	Greenhouse	6	Harvesting	Mature	04 December 2012
E	Pepper	Greenhouse	3	Harvesting	Mature	13 May 2014
F	Cucumber	Greenhouse	3	Harvesting	Mature	04 June 2014
G	Parsley	Greenhouse	3	Harvesting	Mature	18 June 2014
H	Eggplant	Greenhouse	3	Harvesting	Mature	25 June 2014
I	Pepper	Greenhouse	3	Harvesting	Mature	08 July 2014
L	Tomato	Greenhouse	3	Harvesting	Mature	22 July 2014
M		Packaging	2	Wrapping	Mature	10 September 2014
Na	Arugula	Greenhouse	2	Harvesting	Mature	21 October 2014
Nb	Tomato	Greenhouse	2	Harvesting	Mature	21 October 2014
Nc		Nursery	2	Potting	Young	21 October 2014
Oa	Strawberry	Greenhouse	3	Nurturing	Young	04 November 2014
Ob	Outside		3			04 November 2014

are non-allergic. This type of asthma is often referred to as ‘asthma-like disorder or syndrome’ or ‘irritant-induced asthma’ and is highly prevalent in farmers and farm-related occupations and is in these occupations assumed to be caused by bioaerosol exposures (particularly endotoxin)[8]. In addition, airborne organic dust may settle on the leaves during the growth season so that their surface may also be an additional reservoirs of Gram-negative bacteria[9]. Therefore, due to the potential high exposure levels, there is the persistent need to monitor bioaerosol exposure where plant materials are handled.

The main purpose of this study was to measure concentrations of airborne endotoxin in greenhouses with different cultures. The influence of microclimate (temperature, relative humidity, and wind velocity) was studied together with the correlation between endotoxin levels and type of cultured vegetables (broad leaf or small leaf).

Greenhouses

The study was performed in 2010–2014, during the May-December period. The investigation was carried out in South Italy in a company specialized in the production and package of vegetables. Table 1 shows a detailed description of the investigated environments. Growers’ working tasks depend on the growth stage of the crop plants. The main working task included in this study was harvesting of mature crops, but potting, nurturing and wrapping in the packaging department were also investigated.

Sampling and analysis of inhalable airborne endotoxin

Stationary inhalable dust samples were collected using airChek2000 pumps (SKC, Inc., Eighty Four, PA, USA), set a flow rate of 2 l min^{-1}, equipped with IOM sampler and fiber glass filter (GF) (porosity: 1.0 μm) (SKC, Inc., Eighty Four, PA, USA). This type of filters were chosen as they have a high sampling efficiency, low toxicity, and low pressure drop. All air samples were collected at 1.5 m height from the ground and were placed among plants in the passage in greenhouses, as well as in the packing department during wrapping activity and close to irrigation system in nursery.

Airflow was calibrated before and after field sampling with a pocket calibrator (Drycal, Bios International Corporation, Butler, NJ, USA). A 5% change of flow rate was accepted between the two measurements. The volume of air sampled was calculated based on the flow rate and the duration of sampling. On each sampling, day control filters (field blank) were included, which were handled in the same way as the other samples. Air samples were taken continuously for 4 h.

Filters were extracted in 5.0 ml Pyrogen-Free Water with 0.05% Tween 20 by orbital shaking (300 r.p.m.) at room temperature for 60 min and centrifuging (1,000 g) for 15 min. The supernatant was stored at −80°C for 12–24 h to await endotoxin measurement (CEN EN 14031:2003)[13]. Supernatants (in duplicate) were analyzed by the kinetic Limulus Ameboecyte Lisate test (Kinetic-QCL endotoxin kit, Lonza Walkersville, MD USA). To obtain information about possible enhancement or inhibition reactions of the Limulus Ameboecyte Lisate assay (LAL), a replicate of each sample was spiked with an endotoxin standard (CSE, 50 EU/ml) (5 EU/ml final activity). The recovery of spiked samples was in the range recommended; otherwise, the measurement was not included or repeated. A standard curve obtained from an *Escherichia coli* O55:B5 (Lonza Walkersville, MD USA) reference endotoxin was used to determine the concentration in terms of EUs (10.0 EU corresponding at 1.0 ng). The limit of detection was 0.005 EU/ml. The data are presented as EU for cubic meter of the air sampling.

Environmental parameters

During each air sampling experiment, temperature, relative humidity (RH) % and air velocity, were measured using a portable detector Multiple Data Acquisition Type BABUC A (LSI LASTEM).

Statistical analysis

The temperature, relative humidity (T°C and HR%) and endotoxin concentrations were measured as continuous variables. Spearman's test was used to assess the correlation between all the variables measured. The correlation between the concentration of airborne endotoxin and the type of the leaves was assessed by χ^2-tests. The significance level for all tests was $p \leq 0.05$ with degrees of freedom DF=1. For this text, we have first cataloged the plants into broadleaf and small leaf and then dichotomized the concentrations of endotoxin measured. We also performed the Wilcoxon rank sum to compare broadleaf vs. small leaf plant for their ranked levels of endotoxin. The significance level for all tests was $p \leq 0.05$ with $n_a = 7$ $n_b = 7$ (n_a=broadleaf n_b=small leaf).

For regard the endotoxin concentration, Table 2 shows the arithmetic mean, range, median and SD of airborne endotoxin detected in the different sampling points together with environmental parameters. A total of 58 air samples were analysed 3 of which were taken outside the greenhouses within a 5 m distance in the nearby ambient air (Ob) to assess indoor to outdoor (I/O) ratios. In fact, outdoor airborne microorganisms often influence the levels of airborne microbial contamination in indoor environments. The I/O ratio was calculated comparing samples Ob (Outside) with Oa (Inside greenhouse). In this study, the mean I/O value is 2.07. The airborne endotoxins occur in all the greenhouses investigated in low concentrations. Our mean concentration of inhalable endotoxin was 4.37 EU/m^3, with a range of 0.61–27.9 EU/m^3 (corresponding to 0.06–2.79 ng/m^3). In addition, our data show that the concentrations of endotoxin are not correlated with the temperature and relative humidity values (Spearman test: $r_s = 0.066$ $p > 0.05$ for temperature vs endotoxin; $r_s = 0.056$ $p > 0.05$ for relative humidity vs endotoxin for n=20).

In fact, some authors correlated the variability of the concentration of endotoxin to the type of the leaves, and then the type of crop. In our study, we assessed the difference between broadleaf and small-leaf classes for endotoxin levels. The statistical analysis shows a good correlation (χ^2 test: $\chi^2 = 13.99$; $p < 0.001$ degrees of freedom=1; Wilcoxon rank sum test: R=35; $p < 0.05$ for na=7 nb=7), indicating higher endotoxin levels for broadleaf crops, as shown in Fig. 1. The box plot represented in Fig. 1 shows the quartiles of endotoxin concentration for the different type of leaf. The lower end of the box represents the Q1 and the upper one the 3 Q. The lines extending vertically from the boxes (whiskers) indicating variability outside the upper and lower quartiles (upper whisker=3 Q+1.5 r and lower whisker=1 Q−1.5 r; r=3 Q−1 Q). The asterisk represents the outliers.

The results shown in this paper indicate that airborne endotoxins occur in all the greenhouses investigated in low concentrations. At the present time, there are no Occupational Exposure Limits (OELs) accepted by the scientific community to assess the risk of exposure in its entirety. In the absence of occupational exposure limits, our results (mean concentration of inhalable endotoxin 4.37, with a range of 0.61–27.9 EU/m^3) could be compared only to the proposals raised by particular authors or Expert Committees. Referring to the limit proposed by the National Health Council of the Netherlands (DECOS)[14], with regard to the levels of OEL of 5 ng/m^3 (=50 EU/m^3), our results are significantly lower. However, our data are comparable with those reported by Monsò[10] (0.17–0.89 ng/m^3) in the greenhouse workers dealing with flowers and ornamental plants in Spain; but are significantly lower than those shown by Madsen[7] (median: 13.2 EU/m^3) in Denmark, by Radon *et al.*[11] (0.05–12.68 ng/m^3) in Europe, by Adhikari *et al.*[12] (8.20–38.90 EU/m^3) in three greenhouses located in the Midwestern USA, by Spaan *et al.*[6] in cucumber and paprika nurseries where the levels of endotoxin were 36–650 EU/m^3, by Madsen *et al.*[9] who found levels between 0.5 and 400 ng/m^3 in cucumber and tomato nurseries of Denmark. In addition, is known that exposure to dust and endotoxin measured in various professional fields showed that the levels of exposure appear to be dependent on many factors. In agriculture, exposure levels are very different among different farms, crops, seasons and leaf dimensions. In fact, some authors correlated

Table 2. Characterization of investigated environments. Concentration and exposures are presented as arithmetic mean (range), Median (M), Standard deviation (St.D.), not detrermined (ND), Relative Humidity % (RH %), Wind Velocity m/sec (WV m/sec), Endotoxin levels (Endotoxin EU/m^3).

Name sample	Crop	Production environment	Number of samplers	Temperature °C	RH%	WV m/sec	Endotoxin EU/m^3
J	Zucchini	Greenhouse	1	24.5 (24.8–25.3) M 24.2 St.D. 0.54	64.3 (60.7–67.8) M 66.8 St.D. 2.15	0.5 (0.25–0.95) M 0.5 St.D. 0.21	0.6
K	Arugula	Greenhouse	1	ND	ND	ND	4.1
A	Lettuce	Greenhouse	6	24.7 (21.1–30.0) M 25.7 St.D. 3.81	61.5 (50.3–73.9) M 61.8 St.D. 12.45	ND	9.0 (2.20–27.43) M 4.8 St.D. 9.57
B	Chili Pepper	Greenhouse	6	23.3 (21.3–29.3) M 21.5 St.D. 3.14	59.3 (41.8–66.1) M 63.8 St.D. 8.69	ND	7.1 (2.04–16.20) M 5.82 St.D. 5.14
C	Zucchini	Greenhouse	6	21.9 (18.8–25.1) M 20.8 St.D. 2.65	77.3 (72.5–83.4) M 79.3 St.D. 5.31	ND	8.4 (2.5–27.94) M 3.8 St.D. 9.8
D	Chili Pepper	Greenhouse	6	16.8 (10.5–23.1) M 16.8 St.D. 5.1	56.8 (42.5–76.6) M 55.6 St.D. 13.01	ND	3.6 (0.48–10.58) M 3.0 D.St 3.72
E	Pepper	Greenhouse	3	24.4 (22.0–28.7) M 24.2 St.D. 1.2	46.3 (30.65–56.0) M 47.5 St.D. 6.07	0.02 (0.02–0.02) M 0.2 St.D. 0.00	2.5 (2.10–2.76) M 2.61 D.St 0.34
F	Cucumber	Greenhouse	3	24.0 (20.25–28.77) M 24.2 St.D. 1.84	65.6 (55.0–78.8) M 64.0 D.St 5.96	0.3 (0.01–1.47) M 0.34 St.D.0.24	6.1 (2.26–13.25) M 2.76 D.St 6.20
G	Parsley	Greenhouse	3	26.1 (23.63–28.85) M 26.1 St.D.1.0	63.1 (54.7–72.3) M 62.8 D.St 3.09	0.24 (0.01–0.8) M 0.21 St.D. 0.17	3,1 (1.84–4.9) M 2.54 St.D. 1.64
H	Eggplant	Greenhouse	3	30.1 (27.47–33.27) M 30.2 St.D. 1.23	67.9 (42.4–83.7) M 69.0 St.D. 8.12	0.14 (0.01–0.87) M 0.1 St.D. 0.12	5.6477 (2.92–9.94) M 4.0 St.D.3.76
I	Pepper	Greenhouse	3	29.2 (28.31–30.39) M 29.2 St.D. 0.37	50.1 (41.1–59.0) M 50.1 St.D. 3.58	0.2 (0.01–0.84) M 0.16 St.D.0.14	6.1513 (2.15–8.33) M 7.96 St.D. 3.46
L	Tomato	Greenhouse	3	26.0 (24.89–27.54) M 26.0 St.D. 0.48	67.0 (57.8–81.1) M 66.7 St.D. 4.32	0.13 (0.00–0.55) M 0.1 St.D. 0.10	4.0216 (2.66–6.64) M 2.76 St.D. 2.26
M		Packaging	2	29.65 (27.0–32.3) M 29.6 St.D. 3.74	60.15 (49.3–71.0) M 60.1 St.D. 15.34	ND	2.6417 (2.64–2.94) M 2.65 St.D. 0.024
Na	Arugula	Greenhouse	2	23.06 (23.03–24.03) M 23.4 St.D. 0.45	60.44 (59.35–61.5) M 62.1 St.D. 0.98	0.53 (0.12–0.79) M 0.38 St.D. 0.32	2.297 (2.08–2.51) M 2.29 St.D.0.30
Nb	Tomato	Greenhouse	2	22.09 (21.52–22.71) M 22.1 St.D.0.47	70.38 (69.2–72.3) M 70.3 St.D. 1.24	0.16 (0.02–0.28) M 0.11 St.D.0.11	1.9637 (1.34–2.58) M 1.96 St.D. 0.88
Nc		Nursery	2	27.3	66.10	ND	2.7099 (2.47–2.94) M 2.7 St.D. 0.34
Oa	Strawberry	Greenhouse	3	23.7 (20.3–26.2) M 23.8 St.D. 0.73	38.0 (33.8–38.7) M 36.2 St.D. 3.34	0.23 (0.00–1.14) M 0.13 St.D. 0.265	6.91 (10.31–4.02) M 6.65 St.D. 2.61
Ob	Outside		3	17.26 (16.73–17.96) M 17.2 St.D.0.36	63.33 (58.6–70.2) M 63.3 St.D. 2.79	0.20 (0.00–0.90) M 0.2 St.D. 0.23	3.32 (2.14–4.32) M 3.4 St.D. 0.95

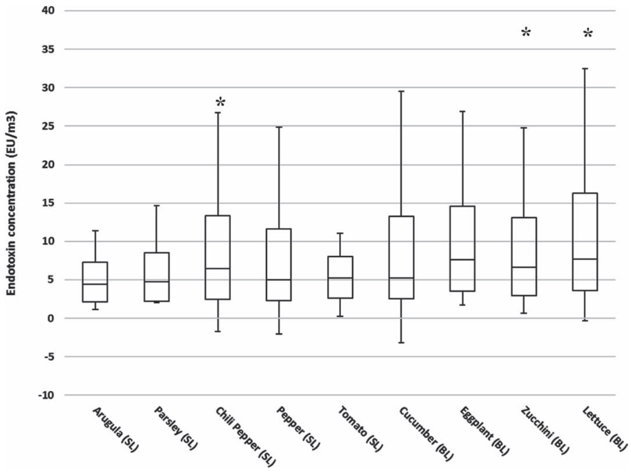

Fig. 1. Shows the concentration of endotoxin found in different types of crops, with broad leaf (BL) and small leaf (SL) in box plot. The samples were classified according to the size of the leaves and named according to the type of crop. The asterisk represents the outliers.

the variability of the concentration of endotoxin to the size of the leaves, and then the type of crop. In our study the results of statistical analysis show that the broadleaf plants were associated with higher levels of endotoxin. This is in accordance with what described by Madsen *et al.*[9], confirming that the broadleaf plants may cause significantly higher exposure of workers of greenhouses to endotoxin.

In conclusion, although endotoxins occur in the air of greenhouse in relatively low mean concentrations, workers engaged in the harvesting and eradication of senescent broadleaf plants can be exposed to higher concentration, even if for short periods, and for these reasons, it is necessary a more careful monitoring of these occupational tasks. Information and training of workers for a proper use of personal protective equipment is always essential.

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