RESEARCH CENTRE FOR INDUSTRIAL CROPS – BOLOGNA (ITALY)

Folsomia candida (Collembola: Isotomidae) Bioassay to Investigate Biofumigation Process by *Brassica carinata* Seed Meals

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Introduction

Biofumigation by pellet based on ***Brassica carinata* defatted seed meals** is beginning to be widely applied in plant defence, also at full field level as a soil amendment.
Starting from the clear biological activity in soil of allyl isothiocyanate (**AITC**) derived from the enzymatic hydrolysis by myrosinase (**MYR**) of sinigrin (**SIN**), the main glucosinolate (**GL**) present in *B. carinata* plants, the aim of this study was to define and develop a new bioassay to test the effect of these meals on springtail *F. candida*.

The Bioassay Method

Folsomia candida (Collembola: Isotomidae) is a widespread microarthropod that occurs in soils throughout the world (Fig.1). It is a parthenogenic species that is easy to grow in the laboratory.
F. candida has been used as a “standard” test organism for more than 40 years to evaluate pollutant effects on nontarget soil arthropods.

The reproduction standard test (ISO 11267, 1999) is not suitable to be applied on biofumigation technique, and an alternative acute toxicity test has thus been planned and developed.
F. candida laboratory culture is maintained at 20±4 °C on a diet of cereal flours.
The substrate utilised in the bioassay is an artificial soil (10% sphagnum peat, 20% kaolinite clay and 70% industrial quartz sand) following Annex V Dir 88/303/EEC, although other substrates such as, for example, agrarian characterised soil can be used. In some preliminary tests the toxicity of AITC was higher in artificial soil than in natural soil, probably due to the soil’s capacity to adsorb active molecules by living edaphic organisms. For these reasons and to increase repeatability, artificial soil was used.

A population of 20±2 living organisms of the same size were taken from laboratory culture and placed in weighing bottles containing 4 grams of artificial soil. 1 ml of deionized water was added to reach optimal moisture for F.candida (Fig. 2). Testing materials can be added both in artificial soil (*e.g.* meals) or in water (*e.g.* vapam®). At least 3 replicates for each concentration have been carried out.
The bottles were immediately closed and placed in a cell at 20± 2°C (Fig.3).

After 24 h exposure, the weighing bottles were opened and mortality assessed by stereo microscope. Since Folsomia floats and walks on water due to water superficial tension, 20±2 °C water was added to simplify the interpretation of results. Substrate in the bottom of the weighing bottles was gently agitated by a probe to allow all organisms to resurface (Fig.4).
Organisms were distinguished between living, dead and, in some applications, living but not mobile (Fig.5).

Mortality %, as regards totality of tested organisms, can be corrected by Abbott’s formula. To accept data, for every single concentration, the coefficient of variation must be lower than 25%.
These mortality results can be processed by probit analysis to calculate the concentration that causes the death of 50% (one half) of tested animals (LC50) or other ecotoxicity values.


Figure 1. *Folsomia candida*

Figure 2. Collecting springtails: (a) laboratory culture, (b) insect aspirator, (c) Petri dish, (d) weighing bottle with aspirator


Figure 3. Weighing bottles in cell


Figure 4. Stereo microscope and probe


Figure 5. Mortality assess

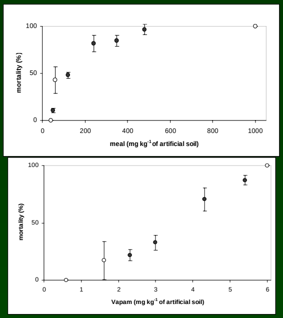
Conclusion

The *F. candida* bioassay seems to be a practical and suitable tool to evaluate the effect of biofumigation on soil dwelling arthropods. Utilising this standardized test, the toxicity of defatted meals from *B.carinata* proved to be around 40 times lower than chemical fumigation by vapam (metham sodium), while the toxicity of the respective active principles produced in soil (AITC and MITC) seemed to be comparable. The toxicity of meals well correlated with GL content, when MYR amount was sufficient to activate the GL hydrolysis.
Recent unpublished studies were directed to better defining the end-point of bioassays and evaluating 48h exposure to further reduce the variability between replicates that are still high, particularly in low effect concentrations.
This bioassay needs to be applied in different conditions to confirm its repeatability and we are willing to provide further information or *F. candida* testing organisms for laboratory cultures.

Biofumigation Application

Defatted Meal *vs.* Metham Sodium

The toxicity of defatted meal was compared with the toxicity of vapam®, a chemical fumigant produced by the Baslini group (Italy) and containing 470 g L^{-1} (39.3%) of metham sodium.
Defatted meals tested in this trial contained 6.99%±0.03 of water, 6.4%±0.1 of residual oil, 6.4%±0.1 of total nitrogen, 158.1 µmol g^{-1}±0.9 of GL (SIN was 97% of total GL) and 17.0 U g^{-1} ±0.4 of MYR. Meal was incorporated in artificial soil and shaken. Vapam was solubilised in water.
Once the toxicity range had been found, LC50 was calculated by probit analysis with fiducial limits (FL) at 95%.
The LC50 of meal was 132 mg kg^{-1} of artificial soil (FL 118-149), the LC50 of Vapam® was 3.4 mg kg^{-1} of artificial soil (FL 3.2-3.6)



Graph 1. Different toxicity of Vapam (metham sodium) and defatted meals from *B. carinata* seeds expressed as *F.candida* mortality mean ±standard deviation. Blank dots indicate data rejected for probit analysis showing a limitation of the bioassay to evaluate low effect concentrations.

Vapam proved to be around 40 times more toxic than *B. carinata* meals.

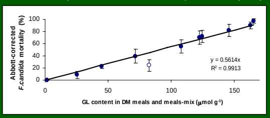
Calculating the recovery yield of SIN in AITC (86%) and metham sodium in methyl isothiocyanate (90%) LC50 was respectively 17.4 µmol kg^{-1} for AITC and 16.0 for MITC.
MITC toxicity was comparable, slightly greater, to AITC toxicity.

GL Content in Meals *vs.* Mortality

The bioassay was also applied on meals derived from different production batches and mixtures of meals with the aim of comparing the toxicity of similar plant materials characterised by different GL contents. The meal concentration applied corresponds to the LC95 calculated in the above reported trial. Four different meals (A, B, C, D), a meal applied after 24 hours watering and drying (D_0) and different mixes of these (A+D,A+3D,3A+D,D_0+A,4D_0+3D,7D_0+D) were tested. All the meals were characterized for GL content by HPLC analysis (table 1).

Meal	Water content (% w/w)	GL (µmol g^{-1}) on DM
A	6.3	88.1
B	6.0	124.6
C	7.4	162.8
D	7.0	165.3
D_0	7.0	1.7

Table 1. GL content observed by HPLC in the different tested meals (A, B, C, D) and expressed in mol on dry matter (DM).

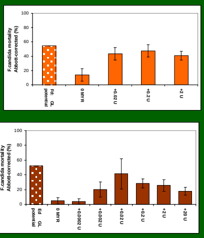


Graph 2. GL content in different *B. carinata* defatted meals and mixes of them *vs. F. candida* mortality.

The different GL concentrations was related with *F.candida* average mortality corrected with Abbott’s formula, showing a linear regression with a very high coefficient of determination (R^2=0.9913).
Only the mortality data of meal A did not fit with the regression curve, probably as a result of the very low MYR content due to high temperature reached during seed oil extraction. On the contrary, when A meal was mixed with other meals, the mortality values fitted perfectly with the regression curve.
Further specific trials were therefore planned in order to better understand the relationship between MYR content in meals and *F.candida* mortality.

MYR Content in Meals vs Mortality

Two different defatted meals (E, F) with a GL content of 121.2 and 109.0 µmol g^{-1} were placed in an autoclave at 256° (E_d) and 121°C (F_d) for 10 minute for total enzyme MYR denaturation. After treatment the GL content was slightly lower: 93.4 and 97.4 µmol g^{-1} respectively.
Different amounts of purified MYR (78 U mL^{-1}) were solubilized in water and added to weighing bottles already containing artificial soil, meal and test organisms. In this case no data were rejected because of high coefficients of variation; in effect variability was in some cases very high as illustrated in graphs 3 and 4.
The meal concentration was the same as used in previous trials (GL content). Potential mortality was calculated starting from the regression line calculated in graph 2. 0 MYR indicates mortality of meals after treatment in autoclave.
The results seem to show that an increment of exogenous MYR can increase the biocidal activity of meals, but over a specific MYR concentration a mortality decrease was observed, in this case utilising *F. candida* mortality and artificial soil as substrate; this value seems to be between 0.02 and 0.2 U mL^{-1} for 2 mg of meals. Other trials are needed to reduce the standard deviation and define an optimal amount of MYR to maximize biocidal effects.



Graphs 3 and graph 4. Mortality due to defatted seed meals treated in autoclave (E_d in graph 3 and F_d in graph 4) to denature MYR, afterwards added as purified MYR to water in different dilutions. Values indicate Unit of MYR mL^{-1} added for every replicate, GL potential is mortality forecast by graph 2. Error bars indicate standard deviation between replicates.

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