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# Environmental contamination from pesticides. Quantification of the response of two fodder plants in presence of pesticides and herbicides in the soil

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***Riassunto.*** *Contaminazione ambientale da pesticidi. Quantificazione della risposta di due piante foraggiere in presenza di pesticidi e diserbanti nel suolo.*
L'uso di pesticidi, naturali o sintetici, per la protezione delle piante dall'attacco di parassiti o piante infestanti, al fine di migliorare la resa colturale delle produzioni agricole è antichissimo. In questo lavoro sono state compiute analisi quantitative su alcuni pesticidi utilizzati per l'irrorazione dei campi con colture arboree, ipotizzando una loro ricaduta sul terreno durante l'irrorazione e l'assorbimento da parte di specie foraggiere. I risultati mostrano un evidente assorbimento da parte delle specie vegetali delle sostanze utilizzate.

**Key words:** Fodder plants, GC analysis, Herbicides, *In vitro* accumulation, Pesticides.

## INTRODUCTION

The use of natural products to prevent plant diseases is very ancient as demonstrated from records in Homer poems. In the past, agricultural communities have been using chemical compounds (arsenic, sulphur, mercury, and copper compounds) or plants infusions (i.e., a tobacco infuse) in order to prevent attacks of parasites, fungi and bugs, or to cure diseased plants. With the advent of synthesis chemistry, modern agriculture has found more support for the improvement of the farming conditions. In fact, beyond chemical manures, the introduction of pesticides, insecticides, herbicides, additives for seed and plant conservation has allowed larger crop production than in the past.

Unfortunately, such employment is often unscrupulous, and the antiparasitic treatments are often carried out in an incorrect and irrational way. All this has caused environmental pollution, with a

direct damage to the environment and, indirectly, to man and animals, final or intermediate consumers of the agricultural products.

Thus, the residues must be easy degradable into non-toxic products, otherwise, employment of non-biodegradable products which can accumulate in living organisms may cause serious consequences on the alimentary chain.

The behaviour of the pesticides in the field depends on four main groups of processes:

- absorption from living organisms with eventual entrance in the alimentary chain;
- adsorption from organ-minerals constituent, with possible consequent release;
- transformation in residual products of various kinds and properties by chemistry or enzymatic reactions;
- loss or elimination from the system for rill, volatilisation, leaching, chemical or biological degradation.

In edible fruit producing trees, the plant treatment typically occurs by using an atomiser and often this procedure of spreading pesticides provokes loss of the product and its dispersion in the environment. Moreover, a part of the atomiser flow can fall back on the field rather than on the epigeous portions of cultivated trees and, by means of rill, it can reach the ground, where herbaceous cultures can be found, as well as forage or vegetables. Also the wind, favouring dispersion on a much greater area, may represent a relevant variable; as a consequence, the presence of pesticides in zones distant from the treated area (flooded crops) is an important parameter, which is not usually held in the appropriate consideration (WANG FREEMARK,1995).

Purpose of this work has been to determine the absorption of pesticides from vegetables, used for animal feeding in livestock farms, not representing target species for the pesticide treatment but accidentally coming in contact with pesticides as a consequence of incorrect plant treatment practices. We have carried out our experiments setting up in vitro cultivation methods and gas chromatographic analyses aiming at the proposal of an experimental model

applicable to different species of vegetables and to various pesticides (WANG FREEMARK,1995)

## MATERIALS AND METHODS

Commercial seeds of *Zea mays* L. and *Avena sativa* L. were used for this work.

The seeds were sterilised as follows: 3 min in EtOH, 20 min in a sodium hypochlorite solution 20 % added with 1 ml of Tween 80. After each step, seeds were washed three times with sterile distilled water (MIGLIORE *et al.*, 1996)

The culture medium used was MS (SIGMA), supplemented with 20g/l of sucrose and 7g/l of agar. The pH of the culture medium was adjusted to 5.5 and sterilised at 121 °C for 20 min.

Commercial products containing active principles used for the tests were: Endo 35 EC-SIPCAM (endosulfan), Rogor L40-SIAPA (dimethoate), Bladan M 20-BAYER (methyl parathion), Lasso Micromix-MONSANTO ITALIANA (alachlor and terbuthylazine). These products are those mainly used in the agricultural farms (MUCCINELLI, 1997)

The culture medium was subdivided into 20 sterile Multipurpose Jars type 390, 390 ml (PBI), 50 ml in each jar, with previous addition of the commercial product filtered through leaking membranes Coastar Ø 0.45 µm, Schleicher & Schuell support.

In every jar we put three seeds of *Avena sativa* and *Zea mays* to verify germination and growth.

The jars with *A. sativa* and *Z. mays* seeds were transferred to a growth room maintained at 22 °C, RH 68%, under L:D 12h:12h photoperiod (about 35 cm from the light source, 4 Philips TLD lamps, 18 W, 33 cm). The light intensity was 80 mE $m^{-2}$ $s^{-1}$ (about 4100 lux); it was measured by a LI-COR quantum sensor LI-190 SB, using the conversion 1klx=19,5 mE $m^{-2}$ $s^{-1}$.

For each species we prepared a stock control.

After approximately twenty days, the plants were collected.

Tab. 1. Summarised results for all the tests

Dimethoate 38% in the commercial product

| **ROGOR L40** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Dimethoate** | | | | | | | | |
| TEST 1 | | | TEST 2 | | | TEST 3 | | |
| | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) |
| Plants | 5.0 | 0.25 | Plants | 7.7 | 0.38 | Plants | 8.0 | 0.42 |
| Medium | 16.0 | 26.66 | Medium | 22.0 | 36.66 | Medium | 29.0 | 48.33 |

Methyl Parathion 19% in the commecial product

| **BLADAN M20** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Methyl Paration** | | | | | | | | |
| TEST 1 | | | TEST 2 | | | TEST 3 | | |
| | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) |
| Plants | 5.62 | 0.28 | Plants | ng | Ng | Plants | ng | ng |
| Medium | 12.8 | 2.13 | Medium | === | === | Medium | === | === |

Tab. 1. (continued)

Endosulfan 32.9% in the commercial product)

| **ENDO 35 EC** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Endosulfan α** | | | | | | | | |
| TEST 1 | | | TEST 2 | | | TEST 3 | | |
| | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) |
| Plants | 5.62 | 0.28 | Plants | 6.79 | 0.34 | Plants | 18.27 | 0.91 |
| Medium | 13.63 | 2.27 | Medium | 95.78 | 15.96 | Medium | 77.28 | 12.88 |
| **Dimethoate β** | | | | | | | | |
| TEST 1 | | | TEST 2 | | | TEST 3 | | |
| | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) | | (ng/µl) | amount (mg) |
| Plants | 9.80 | 0.49 | Plants | 12.56 | 0.63 | Plants | 31.28 | 1.56 |
| Medium | 28.54 | 4.75 | Medium | 144.34 | 24.05 | Medium | 132.51 | 22.08 |

Tab. 1. (*continued*)
Alachlor 171 mg and Terbuthylazine 53.2 mg in the commercial product

| **LASSO MICRIMIX** | | |
|---|---|---|
| **Alachlor** | | |
| TEST 1 | | |
| | (ng/µl) | amount (mg) |
| Plants | 10.49 | 0.52 |
| Medium | 389.0 | 64.66 |
| **Terbuthylazine** | | |
| TEST 1 | | |
| | (ng/µl) | amount (mg) |
| Plants | 2.44 | 0.12 |
| Medium | 73.0 | 12.16 |

Plants and culture medium were stocked separately and frozen at –20 °C (MIGLIORE *et al.*, 1996; 1997)

*Avena sativa*

The commercial products containing endosulfan, dimethoate and methyl parathion are commonly used in fruit growing in the amount of 90-130 ml in 100 l of water.

Assuming 100ml/100 l as average value, we have hypothesised that amounts of 30, 20 and 10% are wasted during the atomising (for the previously explained reasons) and go into the soil below the cultivations.

In this regard, we have prepared three different solutions: 300 µl,

200 µl and 100 µl of commercial product in 1000 ml of culture medium.

*Zea mays*

The Lasso Micromix® is a pre-selective herbicide used for the maize culture and employed directly on the field before the germination of seeds.

A culture medium containing 6ml/1000 ml of commercial product was prepared, considering that the agricultural practice is 600 ml/100 l of water.

*Sample preparation*

Before the quantitative chemical analysis the plants were defrosted and then cut.

*Extraction Methods*

10 g of plants were homogenised in Ultraturrax with 50 ml of acetone; then the homogenate was filtered on Buckner and the precipitate washed twice with 10 ml of acetone. The final volume was exactly to 80 ml.

40 ml of the filtrate (corresponding to 5 g of fresh plants), added with 30 ml of NaCl solution 3%, were extracted twice in a 125 ml separator funnel with two amounts of 25 ml of dichloromethane ($H_2CCl_2$).

The organic extracts, re-united and dehydrated with sodium sulphate ($Na_2SO_4$), were then dried with a rotavapor (depression -600 mm Hg; cooling smoke temperature 0 °C; water bath temperature 30 °C).

The walls of the flask were washed with 1 ml of $H_2CCl_2$ and the extract was transferred on a Florisil column 1 g/6 ml (SUPELCO), previously activated with 3 ml of dichloromethane. The washing of the walls was repeated and the extract was added to the column.

The first millilitre of eluate was discarded; then the collection began and 5 ml of eluent mixture (dichloromethane:benzene:acetone, 10:2:2 v:v) was added. All the eluate was collected (approximately 6 ml) and dried using $N_2$.

50 ml of cyclohexane were added to carry out gas chromatographic analysis (KATHPAL *et al.*, 1975; TADEO *et al.*, 1996, TEKEL *et al.*, 1996).

*Medium culture*

After thawing, 300 ml from each medium were extracted in an ultrasound bath with 50 ml of dichloromethane for two times.

The two extracts, re-united and made anhydrous with sodium sulphate, were evaporated in a rotavapor (depression -600 ml Hg; cooling smoke temperature 0 °C; water bath temperature 30 °C).

The dried extract was resuspended in 50 ml of cyclohexane for the gas chromatographic analysis.

*Gas chromatographic operating conditions*

*Endosulfan, Alachlor:*
Instrument: HP 6890
*Column: Alltech Econo-cap ec-5 (SE 54), 32 m Ø 0.32 mm*
Stationary phase: 1 µm
Carrier: Helium; 30 cm/sec
Injector temperature: 320 °C (0.75 min) 1 µl
Detector temperature: µECD, 370 °C
*Dimethoate, Methyl Parathion, Terbuthylazine:*
Instrument: HP 5880 detector
Column: medium-polar hp5, 30 m Ø 0.32 mm
Stationary phase 0.25 µm
Carrier: Helium; 30 cm/sec
Injector temperature: 250 °C (0.75 min) 1 µl
Detector temperature: NPD, 300 °C
*Operating Conditions (for both gas chromato graphs)*
Oven: 50 °C for 2 min, 50°-150° at 25°/min, 150°-260° at 4°/min for 10 min

Software: HP Chem Station

## Results and Discussion

Before carrying out the gas chromatographic analyses, we calculated the residue active principles after purification on Florisil column, calculating the percentage ratio between the concentration of the standards after and before the passage:

Endosulfan a recovery 70%
Endosulfan b recovery 73%
Dimethoate recovery 92%
Methyl parathion recovery 74%
Alachlor recovery 87%
Terbuthylazine recovery 70%

TEST 1 – 0.1 ml of commercial product in 1000 ml of synthetic medium;

TEST 2 - 0.2 ml of commercial product in 1000 ml of synthetic medium;

TEST 3 - 0.3 ml of commercial product in 1000 ml of synthetic medium.

In all three tests the amounts expressed in mg of medium are referred to 1000 ml and the data are referred to the average of three values deriving from three injections.

The results are summarised in Tab. 1.

These results are preliminary to a more complete and complex study dealing with the implications of the use of pesticides in agriculture, especially concerning forages used in livestock feeding.

From the results of this work it can be inferred that the active principle mainly absorbed from the plants was the endosulfan, with the value of 2.50% obtained in test 3. In the case of dimethoate, we found proportionality in the percentages of absorption of active principle in the plants in the various tests.

The case of the methyl parathion in tests 2 and 3 is very interesting, as we did not obtain the germination of seeds: this may be attributed to the fact that, among all the active principles used in this work, methyl parathion is the only one classified as highly toxic.

In the case of the Lasso Micromix the fresh weight of the "treated plants" was 50% less than the "control" and the plants presented only a small and superficial radical apparatus.

In effect, in order for the herbicidal product to carry out its action, is necessary that rain, at least 10 ml, falls within 15 days from the application; otherwise, it is necessary to take the measure of an aid irrigation. This operation was obviously not carried out in vitro and therefore the obtained results are representative of the just described situation.

It is also obvious that the data obtained here can find application in the construction of a model and/or in the analysis of the residual compounds of pesticides in vegetables cultivated not for commercial use by agricultural companies. Other tests will be carried out in the field, to verify pesticide and herbicide concentrations in the adult plants up to the stage of seed production.

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***Abstract***

The use of pesticides, either natural or synthetic, to protect plants from the attack of parasites or weeds, in order to improve the agricultural productions, is ancient. We carried out gas chromatographic quantitative analyses on the absorption by fodder species of some pesticides used in tree cultivation, assuming a partial fall-back on the soil during atomisation. The results show an obvious absorption of the used substances by fodder plants. The laboratory model may be subsequently applied on field tests, in order to obtain results more correspondent to the real contamination of the plants and data on the environmental contamination of the soil.

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