



European Network for the Durable Exploitation of Crop Protection Strategies

IA3 activity: human resource exchange
SA3.2 sub-activity: foster the participation of research teams
from INCO target countries

ENDURE Grants for INCO scientists

Final activity report

1. Information about researcher and sending partner

Name and surname: ZERMANE NADJIA

Professional status: Lecturer (Maître de Conférences)

Sending partner: Ecole Nationale Supérieure Agronomique (ENSA: ex. INA)

Institute/Department/Research Unit: Département de Botanique

Address: 1 Avenue Hassan Badi, El-Harrach, 16200, Algiers, Algeria

E-mail and phone number of the researcher: n_zermane@yahoo.com / n.zermane@ina.dz

Mobile phone: 00213 551762744

2. Information about hosting partner

Hosting partner: Consiglio Nazionale delle Ricerche

Institute/Department/Research Unit: Istituto di Scienze delle Produzioni Alimentari

Address: Via Amendola 122/O - 70125 - Bari - Italy

Supervisor name: Maurizio Vurro

Supervisor e-mail: maurizio.vurro@ispa.cnr.it

Supervisor phone number: direct phone: +39 0805929331 - mobile phone: +39.339.2200937

3. Information about the visit

Duration: 3 months

Starting date: 20 April 2009

Ending date: 19 July 2009

4. Description of the activities and outcomes

Background and context:

The parasitic weeds *Orobanche* and *Cuscuta* spp. constitute one of the major problems of the agricultural production in large parts of the world. These are obligate parasitic plants that obtain their resources entirely from their host plants, severely suppressing them and even resulting in their death.

Orobanche spp. (also known as broomrapes) occur in parts of all continents, but their problem is most acute in the Mediterranean area, the Middle East and Eastern Europe. The species *Orobanche crenata*, *O. ramosa*, *O. aegyptiaca*, and *O. foetida* are today among the major biotic limiting factors to the production of food legumes such as faba bean, chickpea, pea and lentil, and of vegetables, especially crops of the family Solanaceae like tomato, potato and tobacco. It is estimated that 16 million ha cropping area might be potentially endangered by *Orobanche* spp. in the Mediterranean and West Asia and yield losses of susceptible crops can reach 100% in heavily infested fields.

Cuscuta spp. (known as dodders) are stem and leaf parasites that infect many broadleaf crops, ornamentals, weeds and a few monocot crops. *Cuscuta* spp. are serious pests in forage legumes, especially alfalfa and clover, and plague several important crops including potato, tomato, sugar beet, chickpea, eggplant, onion, melon, citrus and many others. *Cuscuta campestris* is the most widespread species in the genus in the world. Yield losses due to infestation with this parasitic weed were estimated at 50 to 75% in tomato, 70 to 90% in carrot and more than 50% in forage and seed production.

Once a seedbank is established, control of *Orobanche* and *Cuscuta* spp. is extremely difficult, as seeds can remain viable in soil for 20 years or more, and continue to germinate and emerge throughout the growing season. Additionally, the nature of attachment and association between host and parasite requires highly selective herbicides to destroy the parasite without crop damage. However, crop-selective herbicides are not yet available and hazardous fumigants, such as methyl bromide, used for *Orobanche* management has been banned due to environmental risks. Transgenic herbicide resistant crops will allow selective control of *Orobanche* and *Cuscuta*, but none are registered for use in Europe because of concerns about their effects on human health and environment. Accordingly, new control strategies that are safe, environmentally sound and cost effective are required. In this context, the use of compounds of natural origin for the development of natural product-based herbicides could be a valuable additional option in the parasitic weed management.

Objectives:

1. Contribution to the elaboration of environmentally friendly natural herbicides.
2. Help in solving the problem of the most troublesome broomrape and dodder species in cropping systems in Europe and North Africa.

3. Exchange of new ideas, new approaches and methodologies in management of parasitic weeds.
4. Increase knowledge and skills in the area of biological control in general and bioherbicides, in particular, and their use for parasitic weed management.
5. Strengthening ongoing links between the host and home institutions as well as contacts between scientists from both institutions.
6. Development of new opportunities for collaboration with the host institution.

Activities carried out:

1. Organic extraction of dried plant material from 10 Mediterranean plant species.
2. Evaluation of 30 crude extracts (i.e. Dichloro-Methane, Ethyl-Acetate and Methanol/water extracts) on seed germination of *Cuscuta campestris* and *Orobancha ramosa*.
3. Evaluation of the dichloro-methane (DM) extracts for antimicrobial and antifungal activities.
4. Evaluation of DM extracts for antimicrobial activity using Flow Cytometry.
5. Evaluation of DM extracts for herbicide activity towards some broad-leaved weeds and *Lemna minor*.
6. Test for toxicity of DM extracts on brine shrimp (*Artemia salina*) larvae.
7. Partial purification of compounds from effective DM extracts by preparative Thin Layer Chromatography (TLC).
8. Evaluation of more than 100 fractions and sub-fractions for their effect on seed germination of *C. campestris* and *Orobancha* spp. and for their antimicrobial and antifungal activities.
9. Purification by column chromatography of crude extract from one plant with the collaboration of the “Dipartimento di Scienze del Suolo, della Pianta, dell’Ambiente e delle Produzioni Animali, Università di Napoli Federico II, Portici, Italy”.
10. NMR spectral analysis of some active sub-fractions and of one pure compound.

5. Links between visit activity and ENDURE

ENDURE activity:

RA4. Improving the basic understanding of the biology of the crop-pest systems

Sub-activity: RA4.3 Exploitation of natural biological processes

The overall objective of my visit was to develop innovative approaches using host-specific natural compounds from plant origin to be applied as environmentally friendly herbicides to manage the most troublesome broomrape and dodder species in cropping systems in Europe and North Africa. Such objective corroborates the work program of several ENDURE activities aimed at implementation of environmentally friendly crop protection strategies with reduced reliance on pesticides. The research program implemented during the 3-months stay in the hosting institution, led to the selection of several crude extracts and partially purified compounds from 10 Mediterranean plant species with a wide spectrum of biological activities including complete inhibition of the parasitic weeds *Orobancha* and *Cuscuta* seed germination, adverse effect on the dodder seedlings growth and healthiness, herbicide activity towards some dicotyledonous weeds as well as antimicrobial and antifungal activities (Figs 1-4). Hence, about 52 partially purified fractions and sub-fractions exhibited antagonistic activity which means that at least the same number of active pure compounds can be obtained when the complete purification of all the active metabolites will be accomplished. Readily, one pure compound is obtained and will be identified soon. The allelopathic properties of this compound will be evaluated in further laboratory bioassays

to be conducted in the sending institution. Based on our findings, it can be concluded that metabolites from specific Mediterranean plant species can be exploited successfully as “natural product pesticides” for future pest/weed management programs. Their merits as alternatives to synthetic chemicals may constitute a framework for future research axis on which one or more ENDURE activities can focus.

Illustrations



Fig. 1. Effect of crude plant extracts and partially purified fractions on seed germination and seedlings of *C. campestris*. a/ control; b/ complete inhibition of seed germination; c/ reduced growth and necrosis of the seedlings.

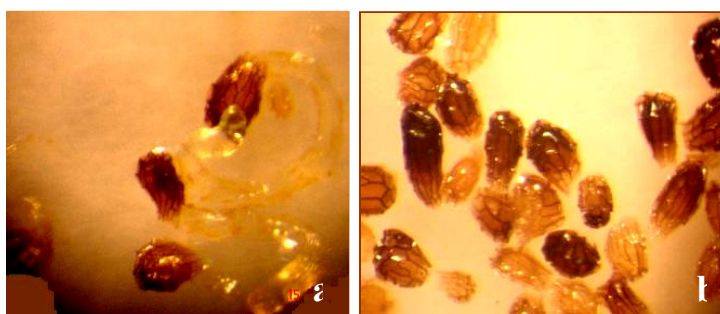


Fig. 2. Complete inhibition of *Orobancha* seed germination after treatment with some extracts (b) compared with the non-treated control.



Fig. 3. Antagonistic activity of some crude and partially purified fractions towards *Bacillus subtilis* (a & b) and *Geotrichum candidum* (c).

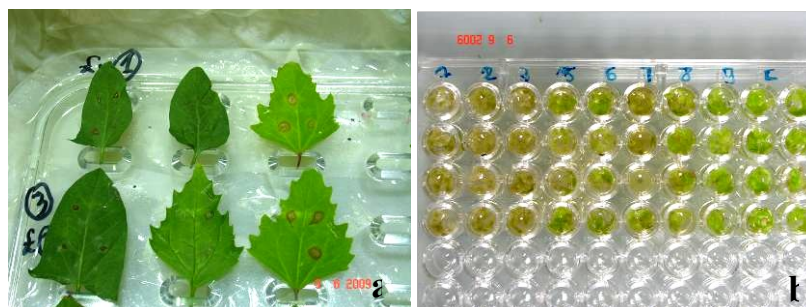


Fig. 4. Effect of crude plant extracts on detached leaves of some Dicotyledonous weeds (a) and on *Lemna minor* (b).

6. Impact

Added value for the researcher:

- **Scientifically**, the stay allowed me to:
 1. Increase my knowledge and skills in the area of biological control in general and natural metabolites, in particular, and their use for parasitic weed management.
 2. Learn techniques used for purification of natural metabolites
 3. Contribute to future development of natural product-based herbicides/pesticides.
 4. Exchange of new ideas, new approaches and methodologies in management of parasitic weeds.
- From a **personal level**, the stay offered to me:
 1. An exciting opportunity to grow personally and professionally.
 2. A continuing contact with European/Italian researchers.
 3. A continuing contact with international issues and programs.
 4. A chance to view one's work and career from different perspectives.

Added value for sending partner and hosting partner:

For the sending partner:

1. Strengthening ongoing links with the host institution as well as contacts between scientists from both institutions.
2. Improvement of knowledge and skills of one of its staff members.

For the hosting partner:

1. Help in capacity building of fellows from developing countries.
2. Development of new opportunities to collaborate with research and academic institutions from developing countries.

Date of submission

05/08/09



Dr. Maurizio Sattin
IA3 activity leader

Approved