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 ICPC target countries**

ENDURE Grants for ICPC scientists

Final activity report

(The form has to be filled in and sent to the activity leader – message should be sent to his p.a. denise.barreiro@ibaf.cnr.it – within 15 days after the end of the visit)

1. Information about researcher and sending partner

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2. Information about hosting partner

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3. Information about the visit

Duration: 04 months

Fist stay:
Starting date: 22 February 2010
Ending date: 22 May 2010
Second stay:
Starting date: 23 September 2010
Ending date: 22 October 2010

4. Description of the activities and outcomes

4.1 Background and context:

Wheat and barley are important cereals in the world. They are highly used for human nutrition. Their cultivation suffers constraints among which diseases are the most important. According to Mathur and Cunfer (1993), Mathre (1997), loose smut, common smut, karnal bunt, blotch and tan spot are diseases of significant economic importance. These diseases adversely affect the production as well as grain quality. The control of seed-borne fungi of wheat and barley is very important as a complementary technology to a good production. Seed health testing and seed treatment using synthetic fungicides such as organo-mercurial fungicides have been used in controlling seed borne fungi inocula (Dharam Vir, 1972; Mishra, 1974).

Despite the success achieved pathogen resistance to synthetic fungicides, the risk faced during the application and possible environmental issues have raised the need of alternatives to synthetic pesticides. Such alternatives are keys issues in the mandate of ENDURE NETWORK. To this effect this project was selected for funding under the external mobility program. This report presents the achievement obtained in Germany under the implementation of this program.

4.2 Objectives:

- Seed health testing of wheat and barley seeds;
- Development of seed treatment based on essential oil extracted from *Thymus vulgaris* for the control of seed-borne fungi recorded in wheat;
- Development of seed treatment based on essential oil extracted from *Thymus vulgaris* for the control - of seed-borne fungi recorded in Barley;
- Evaluation of the seed treatment based on essential oil extracted from *Thymus vulgaris* for its, impact on wheat seeds germination and seedling development.
- Evaluation of the seed treatment based on essential oil extracted from *Thymus vulgaris* for its, impact on Barley seeds germination and seedling development.

4.3. Seed Health Testing of Wheat Seed Samples

A total of 11 wheat seed samples of different cultivars have been tested for seed-borne fungi (Table 1). The samples are from research fields and wheat seed commercial organisation in Germany. Testing was performed following the blotter method described by Singh et al. (1974) and Mathur and Kongdal (2003).

In the tested samples, high level of fungal infection have been recorded especially for the genus *Fusarium;* medium level of infection have been recorded for the genus *Altenaria* and *Cladosporium*; low levels of infections have been recorded for the genus *Bipolaris* in the tested samples(Table 1). The samples identified with high level of infection were selected for the development and evaluation of a seed treatment based on essential oil from *Thymus vulgaris.*

Table 1: Wheat seed samples tested and level of fungal infection detected

Pathogens	% *Fusarium sp*	% *Bipolaris sp*	% *Alternaria sp*	% *Cladosporium sp*
Seed samples				
Apogee	62	0	0	0
Bussard	48	0	2	12
Greina	1	0	4	0
Jacobi	0	0	1	0
HSUN5	0	4	0	0
Mainz	0	0	1	0
Opus	98	0	6	10
Rifmo	92	0	14	12
Tambow	0	0	0	0
Topas	52	0	2	4
Turkis	54	0	14	14

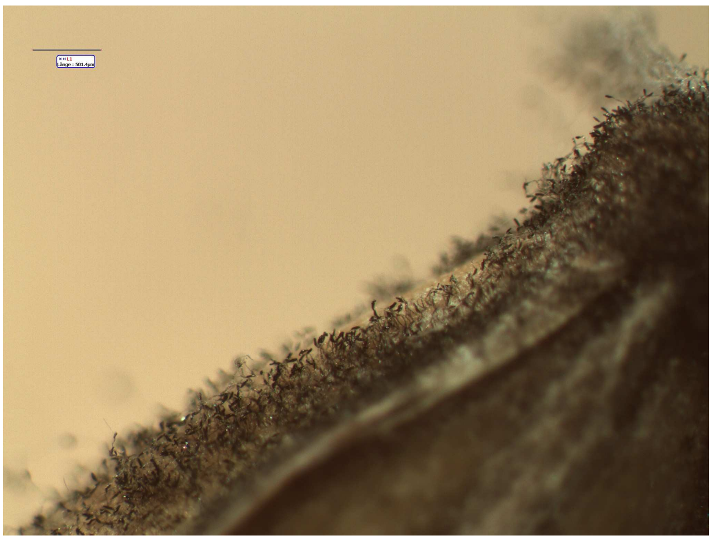

Figure 1: Infection of *Bipolaris sororkiniana* on wheat seed

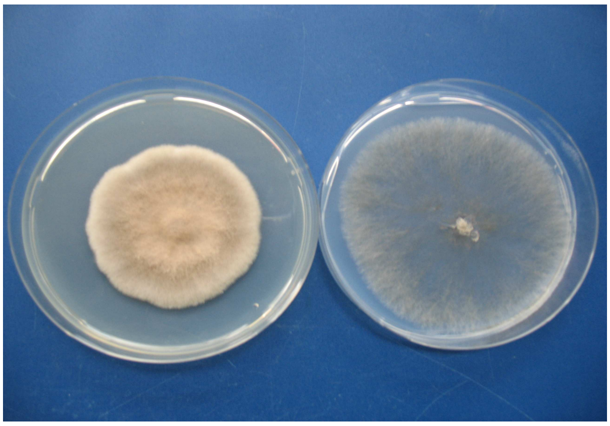

Figure 2: Culture of *Bipolaris sorokiniana* grown of PDA (left) and SNA right

Figure 3: Conidia of *Bipolaris sorokiniana*

Figure 4: Growth of *Fusarium culmorum* on wheat seed

Figure 5: Culture of *Fusarium culmorum* on PDA

Figure 6: Conidia of *Fusarium culmorum*

4.4 Seed Health Testing of Barley Seeds Samples

Two seed samples of barley were tested for seed-borne fungi (Table 2). These samples were of the same variety named DANUTA. They were from organic seed production in Germany. Testing was performed following the blotter method described by Singh et al.(1974), Mathur and Kongdal (2003).
In the tested samples, high levels of fungal infection have been recorded especially for the genus *Bipolaris* (*Dreschlera sp*)*;* medium levels of infection have been recorded for the genus *Altenaria* and *Cladosporium*; low levels and less frequent infections of the genus *Fusarium sp*. have been recorded in the tested samples (Table 2). The sample identified with high level of infection for the genus *Bipolaris* (*Dreschlera sp*) was selected for the development and evaluation of a seed treatment based on essential oil from *Thymus vulgaris.*

Table 2: Seed borne fungi detected in Barley

Pathogens	% *Fusarium sp*	% *Bipolaris sp*	% *Alternaria sp*	% *Cladosporium sp*
Seed samples and variety				
DANUTA sample 1	2	14	26	8
DANUTA sample 2	5	97	12	15

4.5 Testing the Essential oil from *Thymus vulgaris* for its control effects on the *Fusarium* infections in wheat seeds.

Wheat seed identified with high infections of *Fusarium sp* were used. The treatment were 5 different concentration of essential oil emulsified in an agar based solution at the concentration of 12.5%v/v, 10%v/v, 7.5%v/v, 5%v/v and 2.5% v/v.
The treatment was performed by mixing the seeds and the oil emulsion. The experiment included 3 treatment or factors. (1) Treatment with essential oil, (2) Untreated control (3) Control treated with synthetic fungicide ARENA C (Fludioxonyl + Tebuconazole).
Two hundred (200) seeds from the treated were tested for fungi using the blotter method described above. The other haft (200) was evaluated for emergence and seedling development in the greenhouse. The treatments were evaluated in term of reduction in the number of infected seeds on blotter. In the green house the evaluation was based on the number of emerged plants and the total number of seedlings in each treatment after 3 weeks.

Important levels of fungal suppressions were achieved with the essential oil at the concentration ranging from 5 to 12.5% (Table 2, Figure 9). These observations were comparable to the positive control ARENA C (Figure 8).

Table 2: Reduction of the seed-borne fungi in wheat seeds after treatment with the essential oil from *Thymus vulgaris*.

Treatment	Non treated	Arena C	EO12.5	EO10*	EO7.5	EO5	EO2.5
Pathogens							
Fusarium sp	64%	0%	0%	0%	10%	20%	25%
Alternaria sp	50%	0%	0%	0%	4%	24%	30%
Reduction in the infection of *Fusarium sp*	0	100%	100%	100%	85%	70%	55%
Reduction in the infection of *Alternaria sp*	0	100%	100%	100%	92%	52%	40%

EO10= Essential oil at the concentration of 10% v/v

Figure 7: Untreated wheat seed with infection of *Fusarium sp*

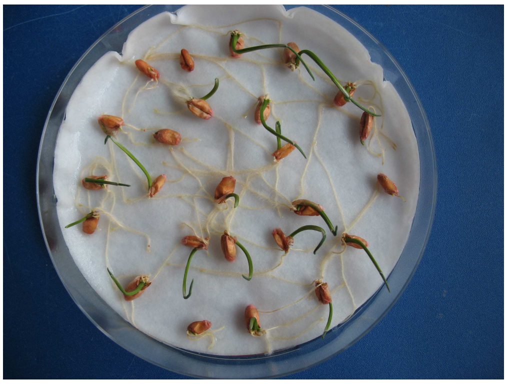

Figure 8: Wheat treated with ARENA C

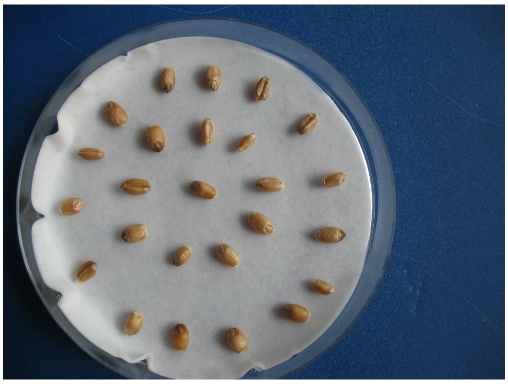

Figure 9: Wheat seed treated with Essential oil from Thymus vulgaris (12.5%)

In the green house the emergence of plant was faster for the untreated seed and the seed treated with ARENA C when compared to that of the seed treated with the essential oil from *Thymus vulgaris*. This delay for the seed treated with the essential oil was a function of the concentration (Table 3).

Despite this delay which disclose a level of phyto toxicity that should be address with other studies we concluded that the solution prepared based on an addition of the agar solution and the essential oil from *Thymus vulgaris* is a potential wheat seed treatment alternative to synthetic chemical.

Table 3: Emergence and plant count of wheat seedling variety Turkis after treatment with essential oil from *Thymus vulgaris*.

Treatment	Non treated	Arena C	EO12.5	EO10*	EO7.5	EO5	EO2.5
Time (days after planting)							
7 days	79%	86%	46%	47%	62%	78%	75%
14 days	75%	86%	62%	66%	69%	81%	72%
21 days	69%	85%	69%	73%	73%	77%	72%

EO10= Essential oil at the concentration of 10% v/v

Figure 10: Seedling from the treated wheat seed (the first and second pot are from seed treated with 12.5% of the essential oil of *Thymus vulgaris*.

4.6 Testing the Essential oil from *Thymus vulgaris* for its control effects on the *Bipolaris (Drechslera sp)* infections in barley seeds.

Barley seeds identified with high infections of *Bipolaris sp* were used. The treatments were 3 different concentrations of the essential oil emulsified in an agar based solution at the concentration of 10%v/v, 8%v/v, 7%v/v and 5%v/v.
The treatment was performed by mixing the seeds and the oil emulsion. The experiment included 3 treatments or factors. (1) Treatment with essential oil, (2) Untreated control (3) Control treated with synthetic fungicide ARENA C (Fludioxonyl + Tebuconazole).

Two hundred (200) seeds from of the treated seeds were tested for fungi using the blotter method described above. The other haft (200) was evaluated for emergence and seedling development in the green house. The treatments were evaluated in term of reduction in the number of infected seeds on blotter. In the green house the evaluation was based on the number of emerged plants and the total number of seedlings in each treatment after 3 weeks.

Important levels of fungal suppressions were achieved with the essential oil at the concentration ranging from 5 to 10% (Table 4, Figure 11). These observations were comparable to the positive control ARENA C.

Table 4: Reduction of the seed-borne fungi in barley seeds after treatment with the essential oil from *Thymus vulgaris*.

Treatment	Non treated	Arena C	EO10*	EO8	EO7	EO5
Pathogens						
Fusarium sp	5%	0%	0%	0%	0%	0%
Bipolaris sp	97%	0%	0%	0%	0%	2%
Alternaria sp	12%	0%	0%	0%	0%	0%
Cladosporium sp	15%	0%	0%	0%	0%	0%
Reduction in the infection of *Bipolaris sp*	0	100%	100%	100%	100%	98%
Reduction in the infection of other fungi	0	100%	100%	100%	100%	100%

EO10= Essential oil at the concentration of 10% v/v

Figure 11: Barley seed treated with the essential oil from *Thymus vulgaris* showing a complete suppression of fungal infection compared to the untreated where heavy growth of *Bipolaris sp*. are observed on the seeds and also on the blotter paper.

In the green house the emergence of plants was fast for the untreated seed and the seed treated with ARENA C. This emergence was delayed for the seed treated with the essential oil and this delay was a function of the concentration (Table 5). Lower emergence was observed from the untreated seed compared to the ARENA C treated seeds and this disclosed the adverse effect of the seed-borne fungal infections on the seedling emergence. Plants obtained from the ARENA C and essential oil treatment were relatively healthier than those from the untreated seeds. As the seedlings from the untreated seeds were developing many of them were dying very probably as a result of the host pathogen interaction (Figure 12).

Despite this delay which discloses a level of phyto-toxicity that should be addressed with other studies we concluded that the emulsion prepared based on an addition of the agar solution and the essential oil from *Thymus vulgaris* is a potential seed treatment for barley. This will be very probably a good alternative to synthetic chemical.

Table 5: Emergence and plant count of barley seedling variety DANUTA after treatment with essential oil from *Thymus vulgaris*.

Treatment	Non treated	Arena C	EO10*	EO8	EO7	EO5
Time (days after planting)						
7 days	70%	81%	7%	33%	50%	66%
14 days	-	-	-	-	-	-
21 days	-	-	-	-	-	-

EO10= Essential oil at the concentration of 10% v/v; (-) data not collected due to time limit

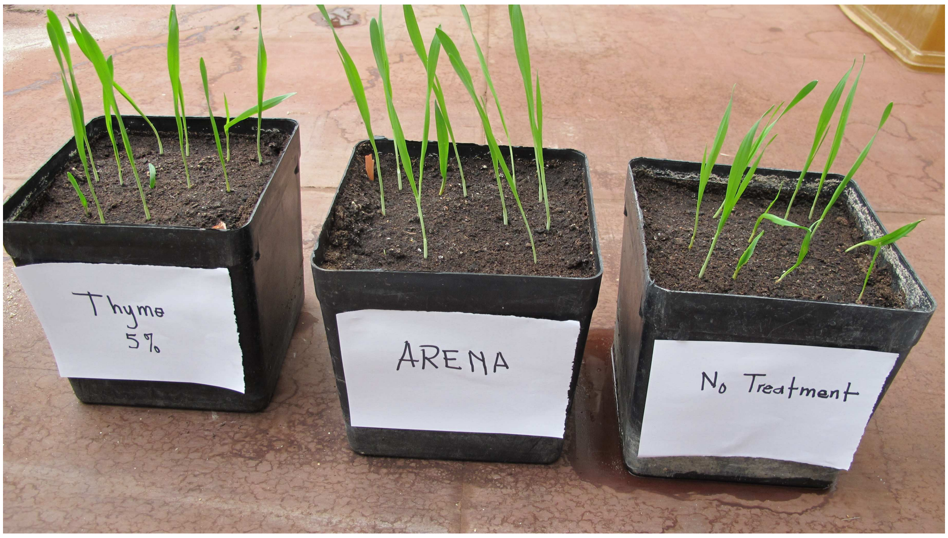

Figure 12: Barley seedling disclosing a relatively healthier appearance of the treated seeds.

4.7 Testing the Essential oil from *Thymus vulgaris* for its control effects on *Ustilago nuda* infections in wheat seeds.

The function of the delayed in the emergence of wheat plants after the application of the seed treatment with essential oil was considered as visible indication of an active interaction between the seed and the oil compounds. It was concluded that this could be studied for its impact on the infection of *Ustilago nuda* which infection is in the embryo.

Such study linking the evaluation of the seed to seedling transmission of the fungus *Ustilago tritici* which is an obligated parasite require the use of a molecular tool such as PCR. This was the reason for Dr Eckhard Koch to recommend Appolinaire for the training on DNA extraction and setting of PCR conditions.

Such knowledge has been acquired during the second stay with the assistance of another scientist Dr Andreas Leclerque of JKI Darmstadt (Andreas.Leclerque@jki.bund.de);

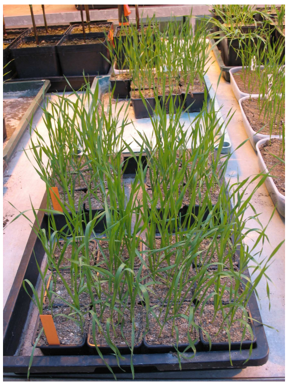

Figure 11: Seedling used for the evaluation of *Ustilago tritici* using PCR

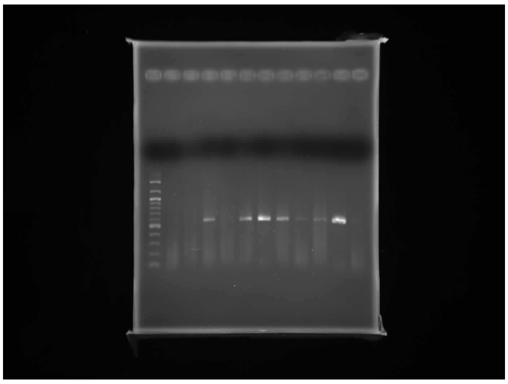

Figure 12: Gel showing Ustilago amplification of DNA from *Ustilago nuda*

5. Links between visit activity and ENDURE

The research achieved falls with the IA3, the ENDURE Human Resources Exchange. It is a contribution to IA1 and especially strengthening the links beyond ENDURE in Europe (Crop Protection Network) and worldwide (INCO countries or Partners Outside Europe (POE)). It is also a contribution to RA4.3 Exploitation of natural biological processes

6. Impact

During the period of the mobility, the scientist had the opportunity to interact with scientist of the plant pathology group at the Biological Institute Darmstadt. He was exposed to various aspects of biological control including the use of *Trichoderma sp* and many bacterial bio control agent. He also had the opportunity to improve his knowledge of DNA extraction and PCR reactions. Such knowledge will also be of significant contribution back at the Institute of Agricultural Research for Development (IRAD) Cameroon.

In addition to the experimental activities, the visiting scientist:

- Participated to many scientific presentation and lectures conducted at JKI Darmstadt, Germany during the period of his stay. This had been an opportunity to mix and learned about the on going of research projects at the Institute of Biological Control.
- He had also be given the opportunity to join scientist of the Institute participating to the annual meeting of the working groups on Mycology and Host Plant parasite interactions' of the German Phytomedical Society held at Konstanz in April 2010. This was an opportunity to meet and interact with German scienstits in a much larger audience.
- He had also been given the opportunity to travel outside Darmstadt to visit others Institutes of JKI, at Braunschweig, Berlin and Kleinmachnow where he discussed and interacted with scientists of disciplines related to this project.

He had also share with colleagues his knowledge of seed pathology and contributed to the improvement of available literature on this topic.

Appolinaire will like to share the lessons learned with other scientists of the west and Central African.

Also he will like to request for further opportunity from ENDURE and other organization to further the investigation on the use of Thyme oil as seed treatment through a joint research activity with research team like that of JKI Darmstadt, Jan Van der wolf of Netherlands and Susana Goggi of USDA who are also active on the same topic.

Date of submission

16.11.2010