

Essential oils main constituents and antibacterial activity of seeds from Iranian local landraces of dill (*Anethum graveolens* L.)

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Abstract In this study essential oils of seeds from eighteen Iranian local landraces of dill (*Anethum graveolens* L.) were chemically analyzed and their possible inhibitory effects were evaluated against Gram-positive and Gram-negative bacteria. The investigated oil was hydrodistilled from air dried seeds and analysed by GC-FID and GC-MS. The screening of antimicrobial activity of essential oil samples was individually evaluated by measuring their diameter of inhibition zone (DD) and minimum inhibitory concentration (MIC) against *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Twenty-seven constituents were identified (≥99.8% of total oil samples) with carvone and limonene, which are as the most abundant compounds that totally constituted about 51.2% to 91.0% of investigated essential oils. Dill apiole (0.2-31.9%), *trans*-dihydrocarvone (3.6-14.5%) and α -phellandrene (0.2-6.6%) were of other components in appreciable amounts. Moreover, the highest activity of *A. graveolens* oils was observed against *Bacillus subtilis* (DD= 27 mm; MIC= 1.87 mg/ml). However, *E. coli* was the most sensitive Gram-negative and, on the contrary, *E. faecalis* was the most resistant Gram-positive bacteria.

Key words

Anethum graveolens L.,
Antibacterial activity,
Essential oil, Carvone,
Limonene

Efficacy of copper fungicides to control potato late blight in organic crop

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Copper fungicides can be highly effective if it is applied prophylactically and with the complete coverage of the foliar surfaces, including the undersides of leaves where the pathogen usually sporulates.

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The results indicated that with regular cheking of the plot, using relative resistant varieties and making a

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potato, late blight,
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Late blight is a plant disease that mainly attacks potatoes and tomatoes, although it can sometimes be found on other crops, weeds and ornamentals in the same botanical family (Solanaceae).

Late blight caused by *Phytophthora infestans* is the most important disease affecting the potato crop in Romania. Late blight is present every year in most of potato growing areas, but not always epidemic. Effective fungicides are required to ensure quantity and quality of yield. Unfortunately, none of the fungicides available today give absolute control of *Phytophthora*. [3]

The first blight successful fungicide was cooper based Bordeaux mixture introduced in 1885 by Millardet [4]. For several decades it was dominant for late blight control in potatoes but its disadvantages conducted to other copper products. So were introduced cooper oxychloride and copper hydroxide

Copper fungicides have contact action, hindering the development of the fungus on the leaf surface.

Today copper compounds are used succesfully only in a complex program, applied early, before the „rows fill in” or towards the end of the potato growing season when the crop is more tolerant to their use [5]. With a very limited number of applications possible, careful monitoring of blight risk is essential.

The key to use these kind of fungicides alone for effective blight control is to prevent infection from occuring by repeated sequential spraying before

infection takes place i.e. during zoospore germination [2].

Copper fungicides can be highly effective if applied prophylactically (before infection) and with complete coverage of all plant foliar surfaces, including the undersides of leaves where the pathogen typically sporulates. In the European BlightMOP project, across many trials in countries across the EU, foliar potato late blight was controlled on average by 27% with an average yield increase of 20% [7]. In a single season field trial at Oregon State University in 2007, four applications of 1.9 lbs elemental copper as cupric oxide suppressed foliar disease development by 88% [8]. In the same time, exist the reverse, in Finland in 2004 was catastrophic, no marketable yield could be harvested, except some early fileds [6]

Materials and Methods

In 2010-2011 field trials were carried out at NIRDPSB Braşov. The field trials consisted of 4 replicates plots in a randomized complete block, 4 rows each with 28 plants.

It was used Sante variety, cultivar with medium resistance to *Phytophthora*. Potato varieties differ regarding their susceptibility. There are varieties with foliar resistance but no tuber resistance. So is important to choose a variety that will allow early harvest such as the tubers escape from the disease.

Planting was made in 28th April 2010 and in 13th May 2011.

Plots are assessed for the extent of blight spots on the leaves. Each plot is assessed as a whole for percentage disease severity using a standard accepted severity key. [1]

Copper fungicides are protectants, so they MUST be applied to the foliage before infection. The copper ion is absorbed by the germinating spore, and the copper denatures spore proteins. Once infection has occurred, copper has no effect on disease progress in the plant.

Effectiveness of spray program was judged in comparison with untreated plot. Dose, volume, method and timing (time of first spray and interval between subsequent sprays in the programme) of application reflect the proposed label recommendations. The interval between sprays usually depends on disease risk. Seven to ten day intervals are used most commonly.

Once late blight is initiated in the field, disease progress can occur very rapidly, killing plant tissues within one or two weeks.

Copper does not degrade in soil and there are serious concerns about the cumulative effect of copper applications on soil copper contents and soil biology. In the European Union, copper fungicides have been

banned completely in the Netherlands and Denmark, and use has been restricted to 6 kg/ha/year (5.4 lbs/A) elemental copper in other EU countries since 2006 [10].

Organic potato producers are restricted in the techniques they can use. So wasn't used herbicides and insecticides to control Colorado beetle, only copper fungicides for late blight, in a maximum quantity of 6 kg/ha/year in accordance with organic crop standards. Field surrounded by trees that shade and slow air movement or those remaining damp late in the morning are at higher risk for infection.

In fields weed control were done only mechanical and Colorado beetle were collected manually.

Observations on foliage were done, in accordance with late blight Protocol. The first assessment was done before late blight apparition. The second assessment was done preceeding the treatment, in the same day. In 2010: 5.07; 12.07; 2.07; 19.07; 26.07 and in 2011: 24.06; 1.07; 11.07; 18.07; 25.07.

Harvest observations done in 13rd September 2010 and 16th September 2011 mentioned the % of tubers with blight and the yield (t/ha).

Table 1

Tested fungicides and rates

Treatment no.	Commercial name	Active ingredient	Dose	A.i. in formulated product (g/kg)
1	Funguran OH 300 SC	copper hydroxide	2,0 kg	600
2	Copernico HI BIO	copper hydroxide 25%	2,4 kg	600
3	Blue Shield 50 WG	copper hydroxide 50 %	2,0 kg	500
4	Alcupral 50 WP	copper oxychloride	3,0 kg	500
5	Control (untreated)	-	-	-

Results and Discussions

Knowledge of enviromental conditions favorable to the occurance and development of late blight attack present special importance in determining the optimal timing of treatments. Key factors for late blight occurence are the weather conditions, like

rainfalls, humidity and temperature during growing season (June – August).

Also important are the inoculum quantity, the distance between source of inoculum and the plants, the plants density and some phisiological aspects that cause a high degree of awareness of the plants.

Table 2 present the meteorological data from the vegetation period in 2010-2011.

Table 2

Main meteorological factors from June to August 2010-2011

Year	Month	Rainfalls/mm				Relative humidity /mm				Temperature			
		DI	DII	DIII	Total	DI	DII	DIII	Average	DI	DII	DIII	Average
2010	June	3,8	48,8	72,4	125,0	74,8	71,8	92,7	79,8	17,2	21,1	15,9	18,1
	July	56,6	57,6	67,8	182,0	88,8	88,0	86,5	87,8	17,4	21,0	19,4	19,3
	August	37,8	0,0	58,4	96,2	89,4	78,6	75,0	81,0	21,4	21,1	17,6	20,0
2011	June	21,0	35,6	54,0	110,6	72,1	84,8	75,3	77,4	18,4	15,8	16,0	16,7
	July	7,2	21,8	24,8	53,8	72,1	73,1	73,5	72,9	18,0	22,0	18,6	19,5
	August	8,2	27,0	0,0	35,2	73,4	79,5	57,5	70,1	18,9	18,4	17,2	18,2

2010 was an year with a lot of rain and high temperatures all the growing potatoes season long. Epidemic phase of the disease was devastating and

quickly installed (late blight appear in July 1st). In July and August rains continued the disease evolutionary

process and accompanied by high temperatures contributed to the foliage drying.

In 2011 from middle of July to the end of the potato growing season rainfalls were increasingly

reduced, which hinder *Phytophthora infestans* development and the capacity of sporulation was restrict.

Mean results for both years are presented in Table 3.

Table 3

Fungicides efficacy and yields - Brasov 2010 – 2011

No.	Product (Commercial name)	Attack intensity* (last assessment)		Yield t/ha		Tubers without blight at harvest (%)	
		2010	2011	2010	2011	2010	2011
1	Funguran OH 300 SC	3,6	4,0	14.28A	18.20A	97.7B	99.7B
2	Copernico HI BIO	3,5	3,5	13.50A	17.20A	97.0B	100.0B
3	Blue Shield 50 WG	3,2	3,8	14.54A	16.40A	97.7B	99.7B
4	Alcupral 50 WP	3,8	4,5	12.85A	16.75A	96.5B	99.5B
5	Control	7,3	6,5	10.13B	11.30B	90.5A	99.7A
	SD (Duncan test)	-		4.398	3.872	2.57	0.32

*Attack intensity:

1 = unattacked;

9 = all leaves dead

In 2010 the attack on foliage was observed for the first time in 1st July. June rains determined to make one preventive treatment. No visible spots on the foliage in that day, but an amount of 125 mm rain and an average of temperature 18°C in June contributed to the installation of epidemic phase of the disease in July.

The first treatment was in pre-infection phase in 28th June. The next treatment was in 5th July when the assesment indicated 10% attack in treated plots and

30-40% attack in untreated (control) plot where most plants are visibly attacked.

The diseases progress quickly under very favorable conditions, humidity 87.8 plus rainfalls in different quantities (from 0.2 to 39.8 mm/day in July) and a temperature average 20°C. After 5 treatments in the epidemic phase of the disease, the attack was between 30-40% and in the control plot (untreated) every plant was affected.

Under climatic conditions in Brasov in 2010 and 2011 all tested fungicides revealed similar efficiency in inhibition of late blight development, without any statistic differences.

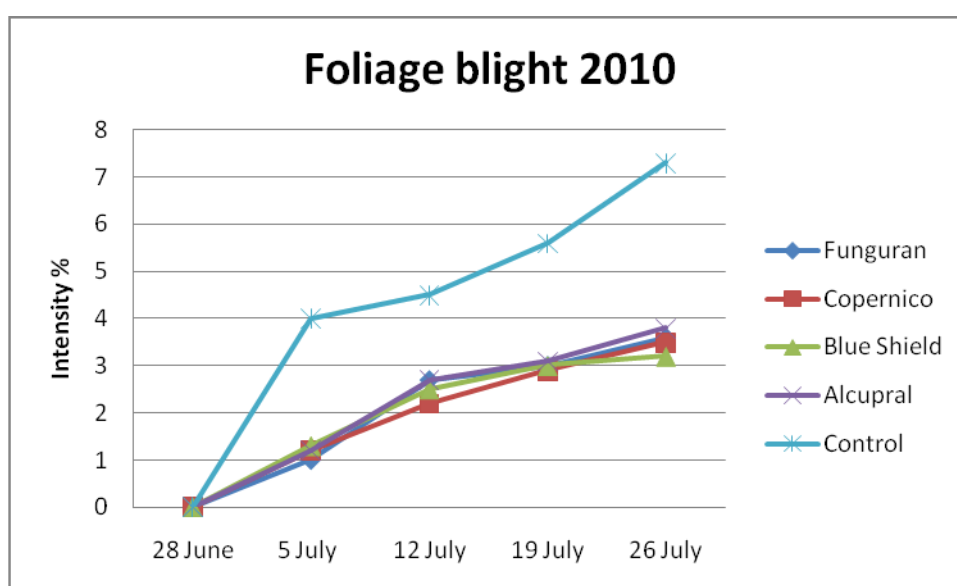


Fig. 1 Development of foliage blight in 2010

In 2011 the first treatment was applied in 17.06, preventive, when no visible spots were on the foliage. But taking into account the climatic conditions the disease was in a incubation phase, manifesting it 4 days later (21th June) when the first spots were observed. Already it was the sporulation phase that get attention to the danger of the disease and the needs for treatments. In the next interval a humidity of 74.4%

plus 54 mm rainfalls and a temperature average 16.0°C influenced the progress of the disease. In July the disease progress was slowly than in 2010 but constant due to the climatic conditions: rainfalls and temperature. From 1st July to 18 July not so much rain but enough to maintain the foliage wet, to create a microclimate for blight(17.8 mm) and a lot of rain 46,8 mm till 25 July.

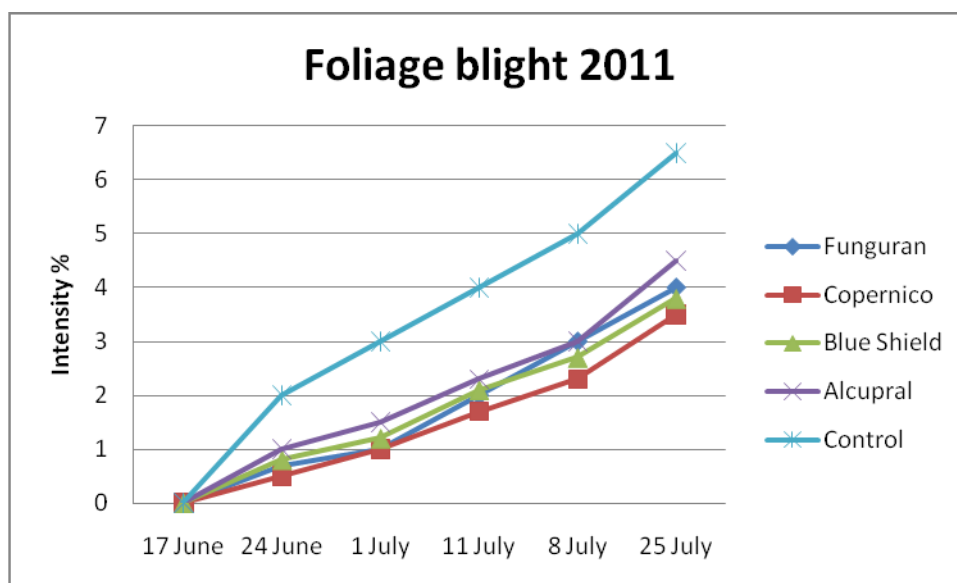


Fig. 2 Development of foliage blight in 2011

2010 was an year with a lot of rain, an amount of 403,2 mm rain and high temperatures all growing potatoes season long. The climatic conditions have influenced the late blight appearance and increasing the attack intensity on foliage, which caused an increased defoliation process.

In 2011 from middle of July to the end of the potato growing season rainfalls were increasingly reduced, only 199,6 mm, which hinder *Phytophthora infestans* development and the capacity of sporulation was restrict. The rainfalls values represented only 53.5-39.2% from the multiannual average.

In both years the disease attack in untreated plot reached a higher level at the assesment time All treatments had significantly less blight on foliage in the end of season compared to the untreated control. Regarding the yield, was statistically similar in all the plots, no differences between them, only the untreated plot has a significant lower production.

In 2011 the yield in all variants was higher than in 2010. Also the tubers contamination (harvest in September) in the treated plots was low, even there isn't a direct correlation between late blight on foliage and tubers late blight. Levels of tuber blight in all

treatments were low, there were no significant differences between treatments.

The cooper fungicides are more succesful when applied as protectant fugicides and also at slowing the development of blight when it's used at an outbreak. They tend to work better when more Cu++ ions are released.

So copper hydroxide formulations like Funguran, Blue Shield work better than copper oxychloride (Alcupral).

Conclusions

Copper is a valuable tool for organic management of potato late blight.

In order to avoid breakthrough late blight are necessary a series of preventive measures, like regular cheking of the plot, for immediate removal, to the first signs, of the blight outbreak.

An organic certified crop must used only certificate seed so it is very important to choose a variety with some level of resistance to late blight to reduce the number of treatments. Early varieties, with rapid bulking rate an early harvest, may avoid the blight epidemy. The high resistance of the tubers to

blight can reduce the risk of crop loss. Also is important to have a good coverage with fungicides of the foliage, especially to protect the new growth in July.

In many areas of the world there are forecasting systems validated to schedule the fungicides applications. It is highly recommended to use them to know the fungicides applications time from point of view of effectiveness, concern for the environment and need for economy.

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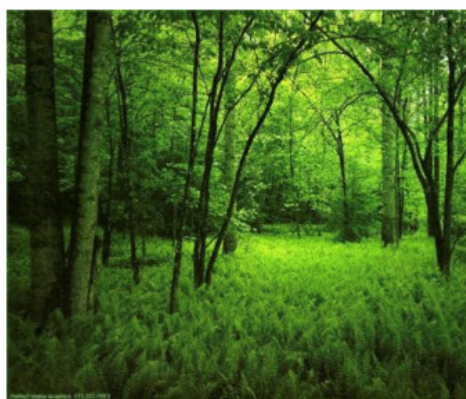
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