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FACULTY OF AGRICULTURE - SKOPJE
REPUBLIC OF MACEDONIA

**DEMONSTRATION PROJECT: ALTERNATIVES TO THE USE OF
METHYL BROMIDE**

FINAL REPORT
ON
BIOLOGICAL AND CHEMICAL ANALYSES
BY CONTRACT NO: 99/197

SUBMITTED JANUARY, 2001

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GENERAL INFORMATION

The main objective of the Demonstration project; Alternatives to the Use of Methyl Bromide in Tobacco and Horticultural Production is to test the following alternative techniques: soilles cultivation, use of low doses chemicals and solarization/biofumigation. In the second phase of the Demonstration project; Alternatives to the Use of Methyl Bromide in Tobacco and Horticultural Production is to adopt the accepted alternatives in the first year i.e. Floating Tray System in the tobacco sector and solarization with biofumigation in the horticultural production. The trials have been placed in production conditions, in the largest companies that are in charge for the production and by out of the tobacco from the individual growers in the region. To illistrate the possible effect of the applied alternative techniques it was neccessary several types of bio-chemical analyses to be performed.

The Faculty of Agriculture along with the laboratories of :

- Institute of Agriculture - Skopje
- Tobacco Institute - Prilep
- Hydrometeorological Department - Skopje

has the performing the required analyses of soil, water and tissue quality, as well as nematodes, fungi and bacteria.

Biological analysis are made as required in TOR, prior to treatment, after treatment and before transplanting. The effectivnes of the treatments was assest with quantitative analisys of the patogens present in the soil and by the appearnace of syphthoms on the plants and roots. Counting for nematodes was carried out under dissecting microscope, and for Fungae, Phycomicetes and Bacteriae the quantitative method for 1 g air dry soil was used with dillution of 1:10; 1:100 and 1:500.

The analyses made have been included as integral part to the all reports submitted by the Agency for Agricultural Development of the Republic of Macedonia to UNIDO

1. WATER ANALYSES

Irrigation water analysis - Prilep:

Analized parameters	Unit	Values
SiO ₂	ppm	1
Redox potential	mW	-111
Conductivity	mS/cm	0.212
m-alkalinity		20
p-alkalinity		0
Free CO ₂	ppm	1.8
Total hardness		6.6
Dry residuals on 105 ⁰ C in		
non filtrated water	ppm	150
filtrated water	ppm	140
Ash residuals in		
non filtrated water	ppm	140
filtrated water	ppm	143
Loss from burning in		
non filtrated water	ppm	10
filtrated water	ppm	7
Suspended matter		
total	ppm	10
organic	ppm	3
inorganic	ppm	7
Noticable		
mechanical residuals		none
color		none
smell		none
pH		8.50
Anions		
Cl ⁻	ppm	7.3
SO ₄ ⁻	ppm	9.1
HCO ₂ ⁻	ppm	122.0
CO ₃ ⁻	ppm	0
OH ⁻	ppm	0
H ₂ PO ₄ ⁻	ppm	0.036
NO ₂ ⁻	ppm	0
NO ₃ ⁻	ppm	0.3
Kations		
NH ₄ ⁺	ppm	<0.01
Na ⁺	ppm	1.4
K ⁺	ppm	0.5
Ca ²⁺	ppm	39.9
Mg ²⁺	ppm	4.5
Total Fe	ppm	0.026
Mn ²⁺	ppm	0.012
Zn ²⁺	ppm	0.113
Cu ²⁺	ppm	0

Irrigation water analysis - Radovis:

Analized parameters	Unit	Values
SiO ₂	ppm	4
Redox potential	mW	-53
Conductivity	mS/cm	0.298
m-alkalinity		19
p-alkalinity		0
Free CO ₂	ppm	8.6
Total hardness		7.3
Dry residuals on 105 ⁰ C in		
non filtrated water	ppm	180
filtrated water	ppm	165
Ash residuals in		
non filtrated water	ppm	168
filtrated water	ppm	160
Loss from burning in		
non filtrated water	ppm	12
filtrated water	ppm	5
Suspended matter		
total	ppm	15
organic	ppm	7
inorganic	ppm	8
Noticable		
mechanical residuals		none
color		none
smell		none
pH		7.06
Anions		
Cl ⁻	ppm	12.2
SO ₄ ⁻	ppm	26.9
HCO ₂ ⁻	ppm	116.0
CO ₃ ⁻	ppm	0
OH ⁻	ppm	0
H ₂ PO ₄ ⁻	ppm	0.003
NO ₂ ⁻	ppm	0
NO ₃ ⁻	ppm	2.8
Kations		
NH ₄ ⁺	ppm	<0.01
Na ⁺	ppm	4.7
K ⁺	ppm	0.8
Ca ²⁺	ppm	33.4
Mg ²⁺	ppm	11.3
Total Fe	ppm	0.139
Mn ²⁺	ppm	0.011
Zn ²⁺	ppm	0.075
Cu ²⁺	ppm	0

Irrigation water analysis - Kumanovo:

Analyzed parameters	Unit	Values
SiO ₂	ppm	3
Redox potential	mW	-41
Conductivity	mS/cm	0.619
m-alkalinity		5.5
p-alkalinity		0
Free CO ₂	ppm	26.6
Total hardness		17.5
Dry residuals on 105 ⁰ C in		
non filtrated water	ppm	370
filtrated water	ppm	358
Ash residuals in		
non filtrated water	ppm	345
filtrated water	ppm	339
Loss from burning in		
non filtrated water	ppm	25
filtrated water	ppm	19
Suspended matter		
total	ppm	12
organic	ppm	6
inorganic	ppm	6
Noticable		
mechanical residuals		none
color		none
smell		none
pH		6.86
Anions		
Cl ⁻	ppm	18.3
SO ₄ ⁻	ppm	24.1
HCO ₂ ⁻	ppm	335.5
CO ₃ ⁻	ppm	0
OH ⁻	ppm	0
H ₂ PO ₄ ⁻	ppm	0.09
NO ₂ ⁻	ppm	0
NO ₃ ⁻	ppm	5.9
Kations		
NH ₄ ⁺	ppm	<0.01
Na ⁺	ppm	5.4
K ⁺	ppm	0.3
Ca ²⁺	ppm	89.1
Mg ²⁺	ppm	22.0
Total Fe	ppm	0.76
Mn ²⁺	ppm	0.007
Zn ²⁺	ppm	0.084
Cu ²⁺	ppm	0

Analysis of weal water in Valandovo

Macroelements (mmol/l)					
NH ₄ ⁺	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Si ⁴⁺
<0.1	0.4	0.9	1.7	1.0	0.33
NO ₃ ⁻	Cl ⁻	SO ₄ ⁻	HCO ₃ ⁻	H ₂ PO ₄ ⁻	
0.2	0.5	0.4	5.4	<0.01	
Microelements (μmol/l)					
Fe total	Mn ²⁺	Zn ²⁺	B ³⁺	Cu ²⁺	Mo ^{s+}
<0.1	0.3	<0.1	34	<0.1	<0.1

EC - 0.6 mS/cm

pH-7.5

Analysis of river water in Valandovo

Macroelements (mmol/l)					
NO_3^-	Cl^-	SO_4^-	HCO_3^-	H_2PO_4^-	
0.2	0.6	0.5	7.2	<0.01	
NH_4^+	K^+	Na^+	Ca^{2+}	Mg^{2+}	Si^{4+}
<0.1	0.3	0.9	2.4	1.1	0.33
Microelements ($\mu\text{mol/l}$)					
Fe total	Mn^{2+}	Zn^{2+}	B^{3+}	Cu^{2+}	Mo^{5+}
<0.1	<0.1	<0.1	24	<0.1	<0.1

EC= 0.8 mS/cm

pH- 8.0

Valandovo - irrigation system

pH in water		Anions (mmol/l)				
	EC mS/cm	NO ₃ ⁻	Cl ⁻	SO ₄ ⁻	HCO ₃ ⁻	H ₂ PO ₄ ⁻
7.4	4.3	16.5	7.1	15.2	0.9	0.09
Kations (mmol/l)						
NH ₄ ⁺		K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Si ⁴⁺
<0.1		3.4	6.8	15.5	8.8	
Mikroelementi (µmol/l)						
Fe total		Mn ²⁺	Zn ²⁺	B ³⁺	Cu ²⁺	Mo ³⁺
0.2		<0.1	<0.1	65	0.9	<0.1

Valandovo -well water

pH	EC mS/cm	Anions (mmol/l)				
		NO ₃ ⁻	Cl ⁻	SO ₄ ⁻	HCO ₃ ⁻	H ₂ PO ₄ ⁻
7.2	0.8	0.5	0.4	0.7	5.8	<0.01
Kations (mmol/l)						
NH ₄ ⁺	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Si ⁴⁺	
<0.1	0.6	0.8	1.6	1.1	0.23	
Microelements (μmol/l)						
Fe total	Mn ²⁺	Zn ²⁺	B ³⁺	Cu ²⁺	Mo ⁵⁺	
<0.1	0.4	<0.1	30	<0.1	<0.1	

Analysis of irrigation water in Prilep

Macroelements (ppm)					
NH ₄ ⁺	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Si ⁴⁺
<0.01	0.4	1.1	40.8	5.6	0.25
NO ₃ ⁻	Cl ⁻	SO ₄ ⁻	HCO ₃ ⁻	H ₂ PO ₄ ⁻	
0.8	8.1	8.3	111.2	<0.01	
Microelements (ppm)					
Fe total	Mn ²⁺	Zn ²⁺	B ³⁺	Cu ²⁺	Mo ⁵⁺
<0.1	0.011	<0.122	12	0	<0.1

EC - 0.201 mS/cm

pH-8.23

Analysis of irrigation water in Kumanovo

Macroelements (ppm)					
NH ₄ ⁺	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Si ⁴⁺
<0.1	0.4	<0.1	25.0	22.0	0.12
NO ₃ ⁻	Cl ⁻	SO ₄ ⁻	HCO ₃ ⁻	H ₂ PO ₄ ⁻	
4.6	20.2	21.8	320.3	<0.01	
Microelements (ppm)					
Fe total	Mn ²⁺	Zn ²⁺	B ³⁺	Cu ²⁺	Mo ⁵⁺
<0.1	0.009	<0.92	30	<0.1	<0.1

EC - 0.812 mS/cm

pH-6.76

Analysis of irrigation water in Radovis

Macroelements (ppm)					
NH ₄ ⁺	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Si ⁴⁺
<0.01	0.9	4.8	38.2	13.8	0.18
NO ₃ ⁻	Cl ⁻	SO ₄ ⁻	HCO ₃ ⁻	H ₂ PO ₄ ⁻	
3.1	10.8	27.8	128.4	<0.01	
Microelements (ppm)					
Fe total	Mn ²⁺	Zn ²⁺	B ³⁺	Cu ²⁺	Mo ⁵⁺
<0.1	0.013	<0.82	31	<0.1	<0.1

EC - 0.359 mS/cm

pH-7.22

2. ANALYSES OF NEMATODES, FUNGI AND BACTERIA

Table 2. Number of colonies in 10 g of air dry soil - cucumbers (during vegetation)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
<i>Fusarium sp.</i>				
Before treatment	19	16	13	14
After treatment	16	6	6	2
During vegetation	11	13	6	32
<i>Trichoderma sp.</i>				
Before treatment	11	10	10	12
After treatment	10	1	7	6
During vegetation	-	5	6	6
<i>Penicillium sp.</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	2
During vegetation	50	3	60	10
<i>Aspergillus sp.</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	-
During vegetation	11	-	-	2
<i>Altrnaria sp.</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	-
During vegetation	4	-	5	-
<i>Phycomicetes</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	-
During vegetation	-	-	8	-

*Saprophytic forms of Bacteria present in all samples

Total nematode density per m² - cucumbers (during vegetation)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
Before treatment	58018	31051	39490	63330
After treatment	212314	4542	28693	36943
During vegetation	57141	6066	10840	22480

S&B-Solarization+Biofumigation

Total nematode density per m²- tomatoe (during vegetation)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
Before treatment	78471	40366	78471	43221
After treatment	366242	2548	28693	21736
During vegetation	220392	6066	3220	4646

S&B-Solarization+Biofumigation

Number of colonies in 1 g of air dry soil - tomato (during vegetation)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
<i>Fusarium sp.</i>				
Before treatment	53	80	49	75
After treatment	13	-	13	8
During vegetation	9	9	1	7
<i>Trichoderma sp.</i>				
Before treatment	10	8	10	9
After treatment	3	4	5	2
During vegetation	-	-	1	12
<i>Penicillium sp.</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	-
During vegetation	43	16	63	-
<i>Aspergillus sp.</i>				
Before treatment	-	-	-	-
After treatment	11	-	-	-
During vegetation	9	3	-	7
<i>Alternaria sp.</i>				
Before treatment	-	-	-	-
After treatment	11	-	-	-
During vegetation	3	-	6	-
<i>Phycomicetes</i>				
Before treatment	2	2	2	2
After treatment	-	-	-	-
During vegetation	-	-	-	-

*Saprophytic forms of Bacteria present in all samples

Total nematode density per m²- cucumbers (year -2000)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
Before treatment	64212	58712	61236	63520
After treatment	8427	6595	4434	3255
During vegetation	9276	7843	8721	5515

S&B-Solarization+Biofumigation

Number of colonies in 10 g of air dry soil - cucumbers (year 2000)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
<i>Fusarium sp.</i>				
Before treatment	35	39	46	35
After treatment	13	10	8	7
During vegetation	14	10	12	18
<i>Trichoderma sp.</i>				
Before treatment	13	11	12	16
After treatment	9	7	4	8
During vegetation	5	9	8	9
<i>Penicillium sp.</i>				
Before treatment	29	28	25	32
After treatment	5	20	12	18
During vegetation	21	15	17	22
<i>Aspergillus sp.</i>				
Before treatment	9	10	12	-
After treatment	-	4	8	-
During vegetation	5	8	7	-
<i>Alternaria sp.</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	-
During vegetation	-	-	-	-
<i>Phycomycetes</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	-
During vegetation	-	-	-	-

*Saprophytic forms of Bacteria present in all samples

Total nematode density per m² - tomato (year 2000)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
Before treatment	79848	55213	59824	62384
After treatment	5487	3359	6686	4900
During vegetation	5763	6422	7727	9132

S&B-Solarization+Biofumigation

Number of colonies in 10 g of air dry soil – tomato (year 2000)

Time of sampling	Control	Methyl Bromide	Dazomet	S&B
<i>Fusarium sp.</i>				
Before treatment	29	49	43	33
After treatment	15	20	28	17
During vegetation	17	29	19	12
<i>Trichoderma sp.</i>				
Before treatment	23	21	30	26
After treatment	12	17	14	14
During vegetation	15	19	18	15
<i>Penicillium sp.</i>				
Before treatment	-	-	-	-
After treatment	-	-	-	-
During vegetation	15	36	25	37
<i>Aspergillus sp.</i>				
Before treatment	15	-	10	9
After treatment	11	2	5	3
During vegetation	7	3	4	7
<i>Alternaria sp.</i>				
Before treatment	7	3	-	-
After treatment	-	-	-	-
During vegetation	5	-	-	4

*Saprophytic forms of Bacteria present in all samples

3. SOIL ANALYSES

Valandovo - greenhouses

pH	EC mS/cm	Anions (mmol/l)				
		NO ₃ ⁻	Cl ⁻	SO ₄ ⁻	HCO ₃ ⁻	H ₂ PO ₄ ⁻
7.5	1.7	5.2	1.2	4.7	1.0	0.05
Kations (mmol/l)						
NH ₄ ⁺		K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Si ⁴⁺
<0.1		1.2	1.8	5.1	2.5	
Microelements (μmol/l)						
Fe total		Mn ²⁺	Zn ²⁺	B ³⁺	Cu ²⁺	Mo ⁶⁺
0.3		<0.1	<0.1	48	0.5	<0.1

Jegunovce - peat analysis (Profile 1)

Depth		0-12	12-32	32-45	45-85
Organic mater %		78.90	81.98	65.11	5.65
Mineral mater%		21.10	18.02	34.89	94.35
pH in water		6.2	6.4	6.1	6.8
pH in KCl		6.0	6.2	6.0	6.4
Available in mg/100 g soil	N	37.97	35.47	26.93	6.20
	K ₂ O	10.0	10.0	12.0	12.0
	P ₂ O ₅	35.20	37.00	25.40	3.60
Total in %	N	2.88	2.47	1.38	0.11
	CaO	4.59	2.46	0.61	4.39
	MgO	3.80	2.36	1.3	3.86
	K ₂ O	0.54	1.40	0.94	0.60
	P ₂ O ₅	0.290	0.178	0.223	0.192

Jegunovce - peat analysis (Profile 2)

Depth		0-20	20-40	40-60	60-100
Organic mater %		63.87	61.35	62.23	9.72
Mineral mater%		36.13	38.65	37.77	90.28
pH in water		5.0	4.1	3.0	5.1
pH in KCl		4.9	3.8	2.8	5.0
Available in mg/100 g soil	N	23.63	20.60	17.30	5.13
	K ₂ O	18.0	12.0	10.8	18.8
	P ₂ O ₅	35.40	31.80	10.60	3.72
Total in %	N	2.31	1.64	1.48	0.25
	CaO	0.58	1.85	0.36	0.64
	MgO	0.78	1.38	0.46	1.64
	K ₂ O	1.10	1.16	0.60	0.94
	P ₂ O ₅	0.131	0.197	0.172	0.296

4. LEAF QUALITY ANALYSES

Chemical analyses of the leaf - Kumanovo 1999

Treatments	Nicotine %	Total N %	N in proteins %	Proteins %	Soluble sugars %	Polyphenols %	Total reduction	Ash %	Schmooks number	Polyphenolic number
C 1	1.29	2.05	0.82	5.12	7.59	2.03	9.62	13.81	1.48	21.10
C 2	1.17	2.08	0.88	5.55	8.87	2.11	10.98	12.40	1.60	19.22
1	0.89	1.43	1.16	7.25	14.47	4.84	19.3	10.21	2.00	25.06
2	1.20	2.58	1.02	6.39	6.23	1.64	7.87	13.62	0.97	20.84

C 1 - Non treated

C 2 - Methyl bromide

1 - Floating tray system

2 - Solarization + Biofumigation

Chemical analyses of the leaf - Prilep 1999

Treatments	Nicotine %	Total N %	N in proteins %	Proteins %	Soluble sugars %	Polyphenols %	Total reduction	Ash %	Schmooks number	Polyphenolic number
C 1	1.59	2.82	1.19	7.45	15.46	3.53	18.99	10.77	2.08	18.59
C 2	2.40	2.14	1.76	10.98	11.72	0.70	12.42	11.73	1.07	5.64
1	1.85	2.72	1.14	7.11	19.17	2.50	21.67	9.62	2.70	11.54
2	1.22	3.54	1.03	6.48	27.69	5.67	33.36	8.99	4.27	17.00

C 1 - Non treated

C 2 - Methyl bromide

1 - Floating tray system

2 - Solarization + Biofumigation

Chemical analysis of the leaf - Radovis 1999

Treatments	Nicotine %	Total N %	N in proteins %	Proteins %	Soluble sugars %	Polyphenols %	Total reduction	Ash %	Schmooks number	Polyphenolic number
C 1	1.59	2.82	1.19	7.45	15.46	3.53	18.99	10.77	2.08	18.59
C 2	2.40	2.14	1.76	10.98	11.72	0.70	12.42	11.73	1.07	5.64
1	1.85	2.72	1.14	7.11	19.17	2.50	21.67	9.62	2.70	11.54
2	1.22	3.54	1.03	6.48	27.69	5.67	33.36	8.99	4.27	17.00

Chemical analyses of the leaf - Kumanovo 2000

substrate mixture		Nicotine %	Total N %	N in proteins %	Proteins %	Soluble sugars %	Polyphenols %	Total reduction	Ash %	Schmooks number	Polyphenolic number
	1	1.57	2.23	1.08	7.76	13.47	3.22	16.69	15.32	1.99	19.29
	2	1.49	2.60	1.04	7.02	13.89	3.52	17.41	14.81	1.97	20.24
	3	1.60	2.44	1.11	6.20	10.18	3.82	14.00	13.44	1.64	21.11
	4	1.52	2.18	0.98	7.11	13.69	3.94	17.69	15.48	1.92	19.81

Chemical analyses of the leaf - Prilep 2000

substrate mixture		Nicotine %	Total N %	N in proteins %	Proteins %	Soluble sugars %	Polyphenols %	Total reduction	Ash %	Schmooks number	Polyphenolic number
	1	1.22	2.05	1.10	7.12	16.35	2.18	18.53	13.12	2.29	18.59
	2	1.17	2.07	0.89	8.14	18.19	2.02	20.21	13.58	2.23	17.02
	3	1.29	2.58	1.16	7.52	15.58	2.31	17.89	14.08	2.07	19.80
	4	1.20	2.70	0.92	7.67	16.22	2.50	18.77	13.72	2.11	18.64

Chemical analyses of the leaf - Radovis 2000

substrate mixture		Nicotine %	Total N %	N in proteins %	Proteins %	Soluble sugars %	Polyphenols %	Total reduction	Ash %	Schmooks number	Polyphenolic number
	1	1.37	2.50	1.07	6.66	14.81	4.68	19.49	13.98	2.22	24.01
	2	1.25	2.58	1.01	6.42	15.15	4.52	19.67	13.21	2.35	23.80
	3	1.42	2.20	1.01	6.51	14.82	4.53	19.35	13.80	2.27	23.17
	4	1.30	2.47	1.10	6.33	14.78	4.27	19.05	13.58	2.33	22.80