

FRIENDLY FOLIAR NUTRITIVE FLUIDS FORMULATION AND THEIR FUNGICIDE CAPABILITY

Marina CÎRJALIU-MURGEA¹, Aura Dana ȘTEFAN², Camelia CRISTEA³,
Viorica CHIȚU⁴, Laurențiu FILIPESCU⁵

Cea mai simplă și eficientă cale de a susține culturile în timpul stadiilor vegetative de dezvoltare constă în utilizarea îngrășamintelor foliare, a biostimulatorilor și a pesticidelor. Cercetarile noastre asupra fluidelor nutritive emulsionate au deschis calea către grefarea funcțiilor fungicide și biostimulatoare în formulările comune de îngrășaminte foliare. Înțelegând particularitățile mecanismului de penetrare foliară și considerând cinetică acestui proces ca un element cheie în formularea fluidelor nutritive foliare, sărurile superbazice de potasiu ale acizilor naftenici și oleici au fost identificate ca cei mai buni componenți ai fazei organice purtătoare a încărcăturii saline conținând micro și macro nutrienții din acest tip de concentrate emulsionate. În lucrările precedente, utilizând testul de susceptibilitate antimicrobiană, au fost cuantificate proprietățile fungicide ale sărurilor superbazice de potasiu ale acizilor naftenici și oleici ca produși intermediari în fabricarea concentratelor emulsionate. În această lucrare, testul de susceptibilitate antimicrobiană a fost extins la toți intermediarii cu conținut de substanțe fungistatice recunoscute și o nouă procedură standard de confirmare a concentrațiilor critice ale entităților cu efect fungicid a fost validată pentru diverse formule echilibrate ale fluidelor investigate.

The most simple and efficient way to handle crop control during any vegetative development stage resumes to the use of foliar fluids fertilizers, growth enhancers and pesticides. Our new formulation approach on emulsified nutritive fluids open ways for grafting additional properties as fungal repellence and growth enhancing to common foliar fertilizes. Apprehending the foliar penetration mechanism and its kinetics as a keystone in formulating foliar nutritive fluids, the overbasic potassium salts of naphthenic and oleic acids were considered as the best choice for the organic phase carrier of the inorganic charge containing macro and micronutrients. Previously, using the diffusion test of antimicrobial susceptibility, we quantified the fungal properties of potassium overbasic naphthenates and oleates as foliar intermediary products. In this paper, the antimicrobial susceptibility test

¹ Eng., Department of Technology of Inorganic Substances and Environmental Protection, University POLITEHNICA of Bucharest, Romania

² Eng., Department of Technology of Inorganic Substances and Environmental Protection, University POLITEHNICA of Bucharest, Romania

³ PhD eng., Department of Chemical Engineering, University POLITEHNICA of Bucharest, Romania

⁴ PhD Eng., Research Institute for Fruit Growing, Maracineni Pitesti

⁵ Prof., Department of Technology of Inorganic Substances and Environmental Protection, POLITEHNICA University of Bucharest, Romania, e-mail: laurentiu_filipescu@yahoo.co.uk

was extended over all the intermediaries dealing with the emulsified nutritive fluids and a new standard operating procedure was designed for assessing the critical concentration of all the moieties bearing fungal properties in the equilibrated formulation of all foliar products under surveillance. Also, the study was expanded to the field products and their effect on the fruits fungal infection during vegetative and harvesting stages, and over the period of fruits normal time storage.

Keywords: fungicide, overbasic naphthenate, overbasic oleate, MIC, critical concentration, storage

1. Introduction

Foliar application of the emulsified nutritive fluids is a new concept designed to increase the efficiency of this intensive practiced treatment in plant growth. There are two distinctive steps in the formulation and manufacture of such products. Intermediary products, which basically are overbasic salts of naphthenic acids or oleic acid carrying growth enhancing and respectively fungicide capacities, were prepared firstly as concentrated stable emulsions. As diluted emulsions these products bring about useful foliar properties in any common nutritive fluid formulas. In the second step, other components as NPK macronutrients with or without supplementary micronutrients, mezzo-nutrients and inorganic fungicides have been grafted on intermediaries under convenient chosen conditions for preservation of the foliar properties and biological activity. According to this concept the new foliar fluids are concentrated emulsions containing two distinctive phases:

- an organic phase which is the carrier of growth enhancing and fungicide functions,
- an aqueous phase yielding all the mineral constituents of usual NPK liquid/foliar fertilizers with or without micronutrients.

Both phases after dilution and hydrolysis are dispatching all the mineral/organic plant nutritive/enhancing entities at a prerequisite level. Stable hydrolyzate emulsions provide high solubility and mobility through waxy cuticle barrier, and capacity to dissolve growth enhancers and fungicide. Aqueous phase provides high soluble saline components carrying macro and micronutrients, additives promoting the stomata opening, low density, viscosity and surface tension and capacity of wetting and spreading as thinly layers over the leaves. All these properties must be associated with typical NPK formulas in foliar nutritive fluids containing growth enhancers and fungicide components [1-16]. The composition of concentrated emulsion can be change in large proportion in terms of component concentrations and component ratio, so the final formulations could be used either as nutritive and growth enhancing or as nutritive and fungicide foliar fluids.

The propose of this paper is extending over three target experiments: a) Test for fungicide effect of each of the major emulsified fluid components (laboratory test); b) Identification of the optimal dosage in order to prevent or to stop the fungal infection (laboratory test); c) Particular fluids application during the entire vegetative season and after crop harvesting (field and storage house experiment).

2. Material and methods

I. Fungistatic test of the major components in the emulsified foliar fluids

Test purpose: a) Evaluation and quantification of potential fungistatic effects of the intermediary products used as independent components in emulsified foliar fluids formulation; b) Optimal formulation of the emulsified foliar fluids on the basis of the fungistatic effect data.

Preparation of the intermediary products used in the emulsified foliar fluids formulation. Potassium overbasic naphthenate and respectively, potassium overbasic oleate, both with molar overbasicity 4/1, were prepared as 1M solutions from analytical pure reactants. Composition of these intermediaries is given in table 1, and their properties are presented in the papers [5-8, 16, 17]. The two stock solutions, taken as fungistatic active intermediary in emulsified foliar fluids formulation were diluted to some individual solutions with the concentration ranging from 10 to 100 mg/L potassium overbasic naphthenate and respectively,

Table 1

Intermediary carrying both the foliar and functional properties in emulsified nutritive fluids

Components	Microemulsion components	Hydrolysed microemulsion components
Potassium overbasic naphthenate		
Potassium hydroxide	R ₁ K, KOH	R ₁ H. KHCO ₃ hydrolysed
Naphthenic acids	R ₁ H	R ₁ H. KHCO ₃ hydrolysed
Carbon dioxide	KHCO ₃	KHCO ₃
Nutrinaft intermediaries		
N1	R ₁ K, KOH, R ₁ H, KHCO ₃	R ₁ H. KHCO ₃ hydrolysed, KHCO ₃
N2	R ₁ K, KOH, R ₁ H, KHCO ₃ , MEA TEA	R ₁ H. KHCO ₃ hydrolysed, KHCO ₃ , MEA·HCO ₃ , TEA·HCO ₃
N3	R ₁ K, KOH, R ₁ H, KHCO ₃ , MEA TEA, CO(HN ₂) ₂	R ₁ H. KHCO ₃ hydrolysed, KHCO ₃ , MEA·HCO ₃ , TEA·HCO ₃ , CO(HN ₂) ₂
N4	R ₁ K, KOH, R ₁ H, KHCO ₃ , MEA TEA, CO(HN ₂) ₂ , (NH ₄) ₂ HPO ₄	R ₁ H. KHCO ₃ hydrolysed, KHCO ₃ , MEA·HCO ₃ , TEA·HCO ₃ , CO(HN ₂) ₂ , (NH ₄) ₂ PO ₄ , KHCO ₃
Potassium overbasic oleate		
Potassium hydroxide	R ₂ K	R ₂ H. KHCO ₃ hydrolysed
Oleic acids	R ₂ H	R ₂ H. KHCO ₃ hydrolysed
Carbon dioxide	KHCO ₃	KHCO ₃

Frucol intermediaries		
F1	KOH, K ₂ S, K ₂ S ₂ O ₃ , K ₂ SO ₃	KHCO ₃ + K ₂ CO ₃ , KHSO ₃ , KHS, S
F2	F1 + MEA/TEA	F1+ MEA·HCO ₃ , TEA·HCO ₃ ,
F3	F1 + R ₁ K, KOH, R ₁ H, KHCO ₃	F1 hydrolysates + R ₁ H. KHCO ₃ hydrolysed, KHCO ₃
F4	F3 + MEA/TEA	F3 hydrolysates + MEA/TEA
F5	F4+ R ₂ K, R ₂ H, KHCO ₃ , EtOH	F4 hydrolysates + R ₂ H. KHCO ₃ hydrolysed + EtOH
F6	F4+ R ₂ K, R ₂ H, KHCO ₃ , EtOH	F4 hydrolysates + R ₂ H. KHCO ₃ hydrolysed + EtOH
F7	F2+ R ₂ K, R ₂ H, KHCO ₃ , EtOH	F2 hydrolysates + R ₂ H. KHCO ₃ hydrolysed + KHCO ₃ + EtOH
F8	F1+ R ₂ K, KHCO ₃	F1 hydrolysates + R ₂ H. KHCO ₃ hydrolysed
Frucol 3	F6 -EtOH	F6 hydrolysates + EtOH
Frucol 4	F7	F7

CO(HN₂)₂-urea; EtOH-ethanol; KOH-potassium hydroxide; KHCO₃- potassium hydrogen carbonate; KHCO₃; K₂S- potassium sulphide; K₂SO₃-potassium sulphite; K₂S₂O₃-potassium thiosulphate; (NH₄)₂HPO₄-ammonium hydrogen orthophosphate; MEA/TEA-monoethanolamine/triethanolamine; R₁H-naphthenic acid; R₁K-potassium overbasic naphthenate 4/1; R₁H-oleic acid; R₂K-potassium overbasic oleate 4/1.

These diluted solutions were used for assigning the minimum inhibitory concentration (MIC) according to a standard test [18], and to the Andrews procedure [19].

Biological material. The inoculums were prepared from standard young strain of *Alternaria spp* and respectively, *Botrytis spp.*, identified on apple tree orchard at RIFG Maracineni Pitesti, Romania, were the field tests for emulsified foliar fluids fungistatic activity were running between 2007 and 2010. Inoculums suspension preparation and inoculums concentration adjustment to 0.4x10⁴- 5x10⁵ CFU/ml, as well as the inoculation and incubation followed the Andrews procedure [19].

Experimental procedure (fungistatic susceptibility test). Diluted intermediaries (potassium overbasic naphthenate and potassium overbasic oleate or their mixtures) solutions having 10 to 100 mg/L concentration have been impregnated on 6 mm Whatman paper discs with a volume of 100 μL solution. For each intermediary product there were prepared at least 10 discs containing 100 μL solutions with the concentration ranging from 10 to 100 mg/L. All the discs were located on the standard Sabouraud culture medium surface in Petri dishes previously inoculated with *Alternaria spp* and respectively with *Botrytis spp.*, and fitted out for tests. Incubation at 25°C lasted 72 hours. After incubation, there were measured the diameter of clean spots formed due to the fungistatic component diffusion around each disc on the surface of culture medium. For *Alternaria spp* there were run only one replica and for *Botrytis spp.*, two replicas.

The runs were set out for 5 workable intermediaries used in the emulsified foliar fluids formulation: potassium overbasic naphthenate (A), potassium overbasic oleate (B), potassium overbasic naphthenate/potassium overbasic oleate mixture 1/1 volume (C), potassium overbasic naphthenate/potassium overbasic oleate mixture 3/1 volume (D) and potassium overbasic naphthenate/potassium overbasic oleate mixture 1/3 volume (E).

II. Optimal dosage identification for foliar application in order to prevent and obliterate the fungal infection

Test purpose: Microbiological analysis and identification of the optimal dosage to remove contamination and end the fungal development.

Preparation of the tested intermediaries. Experiments were run with intermediaries used to produce two types of emulsified foliar fluids: a) **Nutrinaft** formulated as a product promoting foliar nutrition and growth enhancing and b) **Frucol** formulated as a product promoting foliar nutrition and fungi repelling.

These products composition was given in the tables 2 and 3. The state of each chemical compound added to every of the intermediaries from the tables 2 and 3 in both concentrated and diluted products is fully described in the table 1.

Biological material. The inoculums were prepared from standard young strain of *Aspergillus niger* spp., *Fusarium oxysporum* spp. and *Fusarium roseum* spp., respectively.

Table 2

Nutrinaft fluid intermediaries				
Intermediary	Concentration, g/L			
	Potassium overbasic naphthenate	Monoethanolamine/Triethanolamine	Urea	Ammonium hydrogen phosphate
N1	1040	-	-	-
N2	1060	30	-	-
N3	940	30	120	-
N4	807	30	120	133

Table 3

Frucol fluid intermediaries						
Intermediary	Concentration, g/L					
	Potassium hydroxide	Sulphur	Monoethanolamine/Triethanolamine	Potassium overbasic naphthenate	Potassium overbasic oleate	Ethanol
F1	140	30	-	-	-	-
F2	140	30	10	-	-	-
F3	-	30	-	1010	-	-
F4	-	30	10	1000	-	-
F5	-	30	10	970	-	30
F6	-	30	10	930	40	30

F7	-	30	10	823	71	106
F8	140	30	-	-	20	59
Frucol 3	-	30	10	960	40	-
Frucol 4	-	30	10	930	40	30

Experimental procedure (optimum active component concentration test). For an accurate measurement of the optimal active components dosage, the intermediary products under surveillance have been introduced into composition of the culture medium. Thus, in the Czapek-Dox medium, before casting into Petri dishes, there were added variable concentrations of the intermediaries from tables 2 and 3, ranging from 50 and 6000 mg/L. Then, standard young strain of fungi were inoculated on the previously prepared Petri dishes, representing the samples of Czapek-Dox medium blotched by variable concentrations in antifungal products (tables 2 and 3). Inoculation, identification and fungi/yeasts specimen confirmation were made according to Brock recommendation [20]. All experiments were performed during 2003-2008 period.

3. Results and discussion

I. Fungistatic susceptibility test. Antifungal activity of the intermediary products, potassium overbasic naphthenate and potassium overbasic oleate, seems to be certain on *Alternaria spp.* starting up from 50mg/L, but the real eradicating effect has to be placed around 100 mg/L, when the clear spot diameter lays close to 20 mm as figure 1 shows.

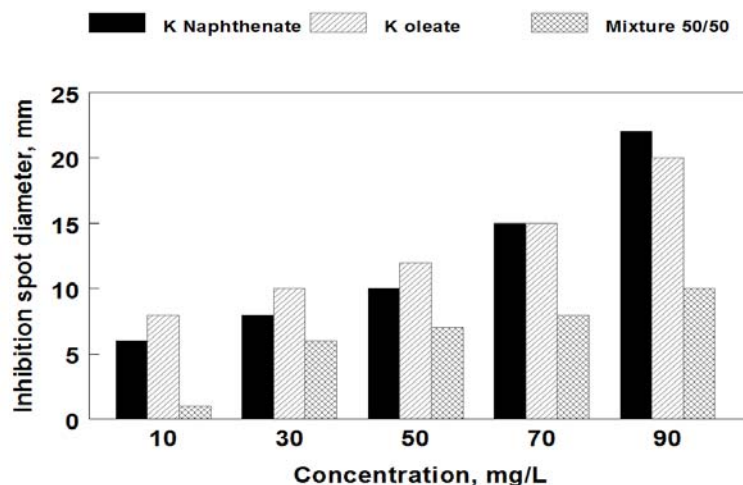


Figure 1. Diameter (in mm) of the inhibition zone covered by the fungistatic intermediary products versus their concentration component diffusion. *Alternaria spp.*

Even if some inhibition forms of sporulation are visible at 10 mg/L, only above 100 mg/L both products inhibit the birth and development of this fungus (figure 2). Such a large margin of safety taken for an unambiguous delivered effect is largely justified by the fact that both products are surely acting through contact mechanism and no other way of inflicting a degenerative process can be assumed. The strength of the overbasic oleate is a little milder, but each one of the two products may replace the other in particular formulations. The mixtures of two intermediary products exhibits faraway less strength excepting the molar ratios potassium overbasic naphthenate/potassium overbasic oleate higher than 3/1, where may be potassium overbasic naphthenate is prevalent in its repellence against *Alternaria spp.* (figures 1 and 2). As far as this test shows, for the mixtures of two intermediary products MIC concentration has to be considered higher than 100 mg/l.

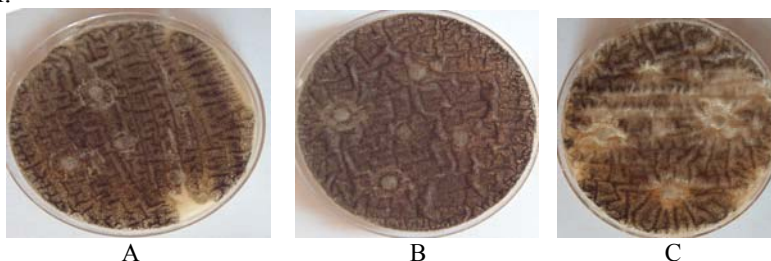


Fig. 2. Diffusion spot in *Alternaria spp.* stained culture media. A. Potassium overbasic naphthenate/Potassium overbasic oleate 25/75; B. Potassium overbasic naphthenate/Potassium overbasic oleate 50/75; C. Potassium overbasic naphthenate/Potassium overbasic oleate 75/25;

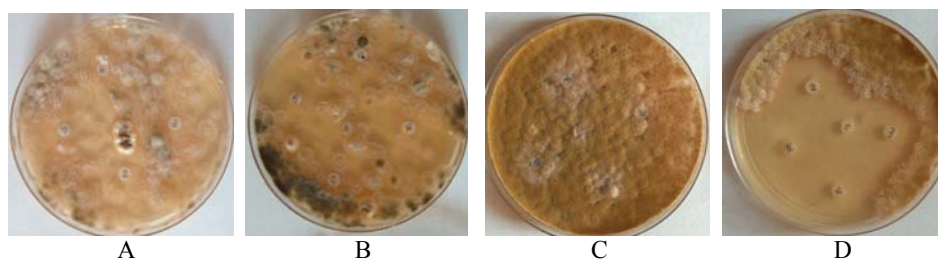


Fig. 3. Diffusion spot in *Botrytis spp.* stained culture media. A. Potassium overbasic naphthenate/Potassium overbasic oleate 25/75, Replicate 1; B. Potassium overbasic naphthenate/Potassium overbasic oleate 75/25, Replicate 1; C. Potassium overbasic naphthenate/Potassium overbasic oleate 25/75, Replicate 2; D. Potassium overbasic naphthenate/Potassium overbasic oleate 75/25, Replicate 2;

Anyway in this case, lesser concentrations on all assay plates prevent the sporulation and hold on the vegetative growth. Potassium overbasic oleate and the mixtures potassium overbasic naphthenate/potassium overbasic oleate are ranking higher on pH scale. Surprisingly, their strength in eradicating *Alternaria spp.* is poorer. Factually, this conclusion seems to deny the known theory of alkalinity

sustaining antifungal properties of potassium compounds like carbonates or hydrogen carbonates [21, 22].

In the case of *Botrytis spp* fungi, only potassium overbasic naphthenate act as a compelling fungicide at concentration up to 100 mg/L. All combinations between potassium overbasic naphthenate and potassium overbasic oleate failed to exert any fungistatic activity, because overbasic naphthenate concentration in any mixture from figure 3 lies below the critical threshold. Nevertheless, the susceptibility test has only a limited validity. Its results are substantiating the tangibility of a real fungistatic effect. Consequently, the true threshold concentration of the active intermediary in any nutritive foliar product has to be measured with another complimentary method to the susceptibility test.

II. Optimal active product concentration

Two nutritive foliar products and all the intermediary components which made up their composition and brought in the optimal foliar, nutritive and fungicide properties have been analyzed in order to find out the real critical threshold concentration, where the fungistatic effect is obviously exerted. The first product, Nutrinaft, is a dual action product, inducing mainly foliar nutrition and growth enhancing [8, 12, 16]. The second product, Frucol was designed as a fungicide, but it is carrying additionally nutritive and growth enhancing capabilities [6, 7]. The fungistatic potency of the main components, potassium overbasic naphthenate and potassium overbasic oleate, was qualitatively demonstrated in the previous study concerning the fungistatic susceptibility test. The critical threshold concentrations were evaluate on 3 young stains of fungi, *Aspergillus niger*, *Fusarium oxysporum* and *Fusarium roseum*, commonly found in the apple three orchards. The accuracy of this test is provided by the fact the fungi inoculums are properly applied on the surface of a homogenous stained Czapek-Dox agar culture media with the studied products at total concentrations ranging from 50 to 6000 mg/L.

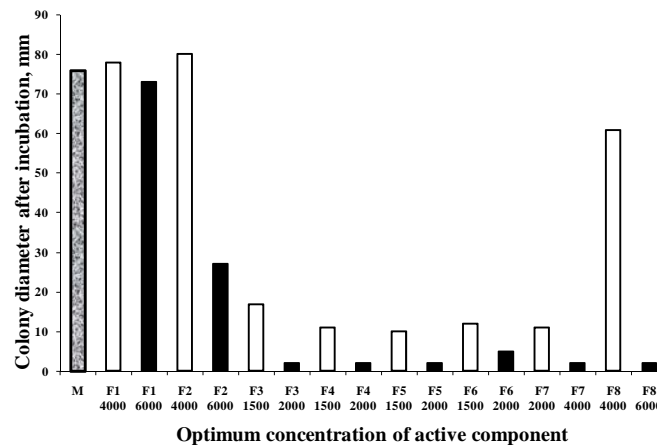


Fig. 4. Fungi colony diameter versus Frucol intermediary products concentration F1 – F8 (table 1). *Aspergillus spp.*, pH 7.

The results of the measurements are given, in terms of colony diameters of the inoculums growths versus the staining products concentration in the culture media, in the figures 4-6 for Frucol intermediary products and in the figures 7-9 for Nutrinaft intermediary products. All the data concern the measurements at pH 7.0. Additional information about the critical threshold concentrations at pH 10 are displayed in the table 4. As it was expected from preliminary data, not all the intermediaries act as fungicides at rational concentration in the range of the customary allowed foliar concentrations. Also, the raise in pH over the value 10 does not decrease significantly the critical threshold concentrations, excepting just some very particular cases (table 4).

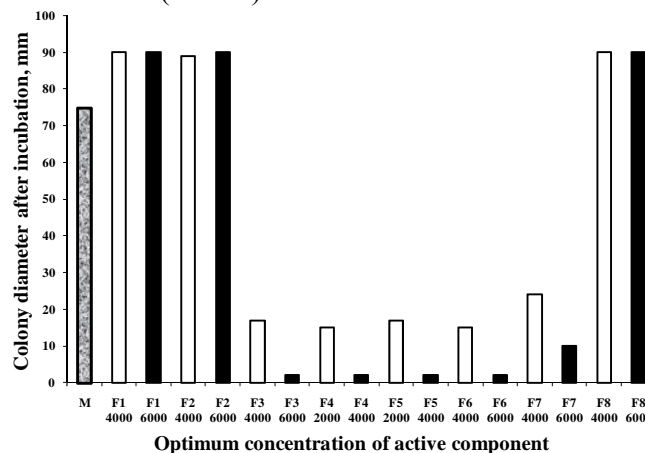


Fig. 5. Fungi colony diameter versus Frucol intermediary products F1 – F8 (table 1). *Fusarium oxysporum spp.*, pH 7.

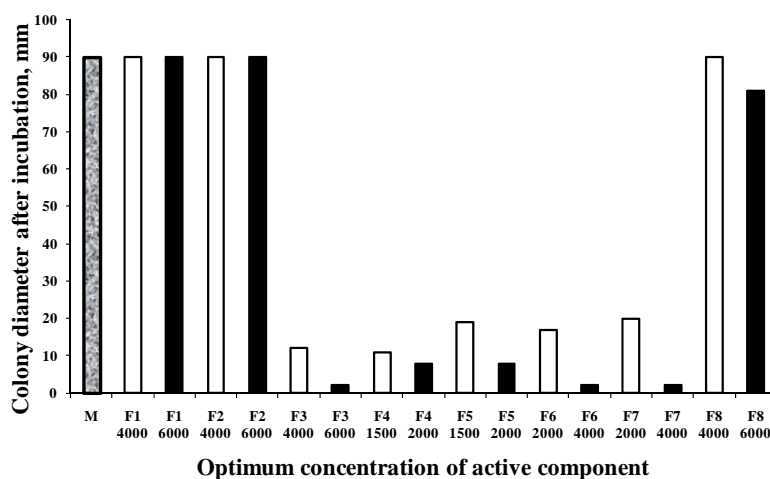


Fig. 6. Fungi colony diameter versus Frucol intermediary products F1 – F8 (table 1). *Fusarium roseum* spp., pH 7.

Each intermediary in the both products shears a specific duty in the multifunctional composite properties. Some contribute to building up of the foliar properties some others bear the moieties involved in nutrition, growth enhancing and fungistatic effects. The above data ascertain not only the strength in each component fungicide capacity, but also the multifunctional contribution to other controlled properties and biological expected resources of the composite nutritional fluid. The knowledge of full contribution of each intermediary is a meaningful tool in the formulation and product formula optimization in order to boost its quality and efficiency.

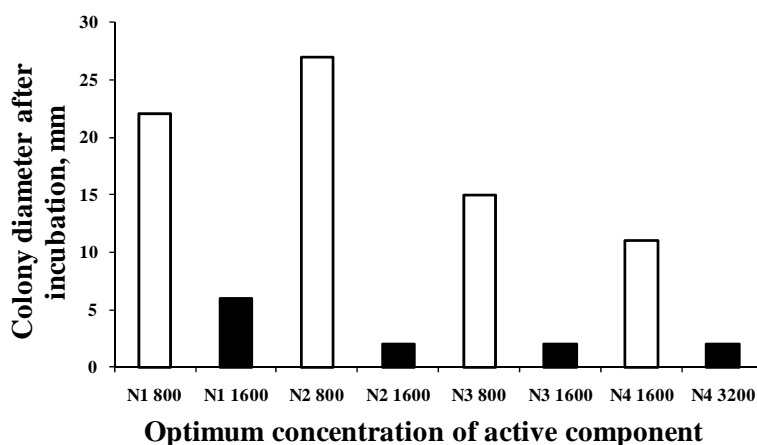


Fig. 7. Fungi colony diameter versus Nutrinaft intermediary products N1 – N4 (table 1). *Aspergillus* spp., pH 7.

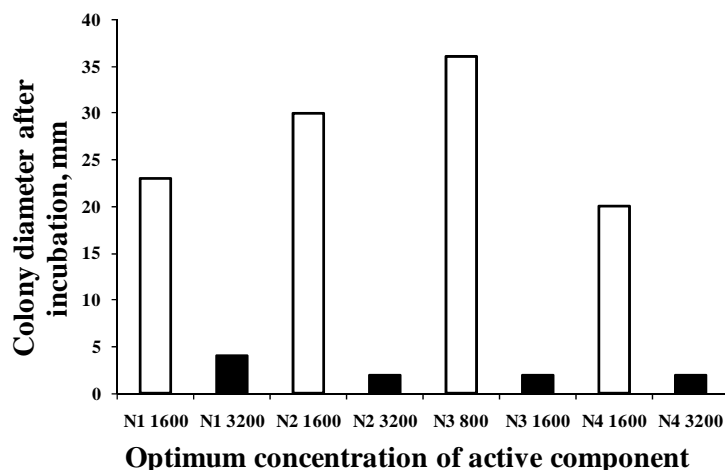


Fig. 8. Fungi colony diameter versus Nutrinaft intermediary products N1 – N4 (table 1). *Fusarium oxysporum* spp., pH 7.

The intermediary F1 is a mixture of potassium sulphides, sulphites, thiosulphates and colloidal sulphur accompanied by their hydrolysis products. The same components are in the intermediary F2, which also contains supplementary added of mono and triethanolamines and their carbonation compounds as adjuvant in foliar properties control. Both intermediaries are fully inactive as fungicide, in spite of their alkalinity and sulphur content. As a matter of fact, in the range of studied concentrations, both intermediaries are completely neutral and allow the inoculums growth and spreading with the same rate as in the control sample (M, in the figures 4-6). The intermediary F3 comes from the partial replacing of potassium hydroxide in the intermediary F1 with potassium overbasic naphthenate. Thus, the new composition, containing a homogeneous mixture the former potassium sulphides, sulphites, thiosulphates and colloidal sulphur, beside the new brought components potassium overbasic naphthenate and its hydrolysates, acquired new al chemical potency and full capacity to repel the growth of all the tested fungi. Because its repellency started below 2000 mg/L and its fungistatic effect might cumulate the sulphur activated contribution, this product could be itself considered a strong fungicide. In the intermediary F4, the entire F3 composition is strengthened by mono and triethanolamines and their carbonation compounds which are good solvents, emulsifiers, growth enhancing and also in-depth contributors to the improved foliar properties.

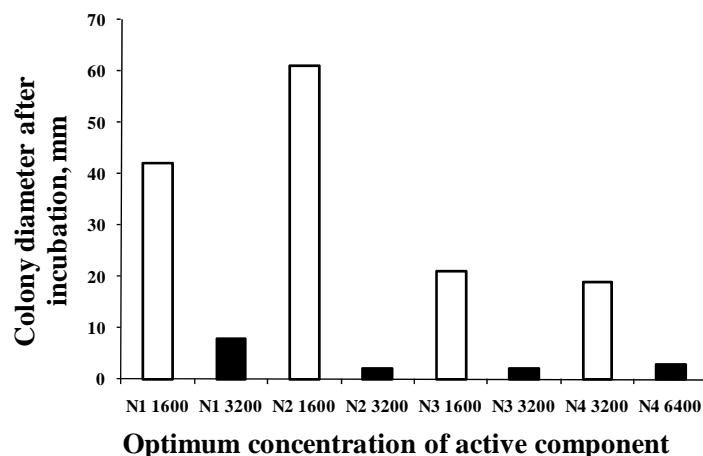


Fig. 9. Fungi colony diameter versus Nutrinaft intermediary products N1 – N4 (table 1). *Fusarium roseum* spp., pH 7.

Table 4

Optimum products concentration for full fungi repelling, ppm concentrated emulsion

Products	pH 7			pH 10		
	<i>Aspergillus niger</i> spp.	<i>Fusarium oxysporum</i> spp.	<i>Fusarium roseum</i> spp.	<i>Aspergillus niger</i> spp.	<i>Fusarium oxysporum</i> spp.	<i>Fusarium roseum</i> spp.
F1	Over 6000	Over 6000	Over 6000	6000	6000	6000
F2	Over 6000	Over 6000	Over 6000	6000	6000	6000
F3	6000	6000	6000	1500	4000	6000
F4	2000	4000	2000	2000	4000	1500
F5	2000	4000	2000	1500	6000	2000
F6	2000	6000	4000	2000	6000	600
F7	4000	6000	4000	4000	6000	4000
F8	6000	6000	6000	6000	Over 6000	6000
N1	1600	3200	3200	1600	3200	3200
N2	1600	3200	3200	1600	3200	3200
N3	1600	3200	1600	800	1600	1600
N4	3200	6400	3200	3200	3200	3200

This new intermediary repellency is as good as the repellency of the F3 product, but its foliar properties are certainly better. Intermediary F5 is coming from the mixing F4 hydrolysis products with potassium overbasic oleate (mild fungicide properties) and EtOH (solvent and properties control additive). This binary product, encompassing as presumptive active components the two overbasic salts, is a more environmental friendly product, whose fungistatic activity is similar to the former intermediary F3 and F4 (figures 4-6 and table 4), but its mild interaction with the plants might be preferred in the case of sensitive plants or cultures. The intermediaries F6 and F7 belong to the same class of

environmental friendly products as the product F5. They differ due to their variable potassium overbasic oleate/potassium overbasic naphthenate ratios, and consequently, due to their mildness. Nevertheless, the contribution of the mild components is limited. So far, the missing of potassium overbasic naphthenate from the composition of intermediaries F5- F7 leads to the product F8, which might provide nutrition and growth enhancing moieties, but the fungistatic effect is merely lost (Figure 4-6 and table 4).

Thus, it was demonstrated that the same range of effectiveness in fungal repellency could be achieved not only by the dosage of the stronger fungicide component, but also by careful and rational formulation. The above analysis could be taken as a basis for formulation multifunctional friendly chemical products used in plant nutrition and crop protection. It seems the pH of different formulations does not interfere with fungistatic potential of the active moieties and does not modify in a clear manner the critical concentration thresholds.

Case of the product Nutrinaft provides a similar approach and tackling. This product was formulated as growth enhancer due to well demonstrated of this property for its main component, potassium overbasic naphthenate [9, 12, 15]. Because the concentrated solutions potassium overbasic naphthenate had to be diluted and neutralized, their reformulation as foliar fertilizers was considered as a helpful path to the product economical design as dual nutritive fluid. Potassium overbasic naphthenate demonstrated capacity to repel fungi open new ways in formulation and use of these fluids. Some intermediary products have been formulated in order to optimize the formula and to improve the product multifunctionality (table 1). Figures 7-9 and table 4 display the results of researches concerning the optimal active product concentration of the intermediaries N1-N4 used as fungicides. Also, the figures 7-9 back up and unveil in a relevant way the capacity of potassium overbasic naphthenate to convey its fungistatic potential to any saline solution. Thus, chemical potency and fungistatic potential of the intermediary N1-N4 products are insubstantially affected by large significant changes in their saline composition. These products critical active concentration does not depend on the solution pH.

4. Conclusions

Emulsified activities, beside their unusual nutritive and growth enhancing capacities, have additionally exhibited several fungicide functions. Using the antifungal susceptibility diffusion test as validation method, there was demonstrated the main components of the Nutrinaft and Frucol activities are certainly acting as mild fungicides. The minimum inhibitory concentration for both potassium overbasic naphthenate and potassium overbasic oleate with the overbasicity 4/1 lay in the 80-100 mg/L. The acting mechanism of both

intermediary products does not overlap or interfere by any means. The trial showed that each product is carrying out its own effect, even if both are contact fungicides. Thus they can be replaced each other or mixed for extending the area of repelled fungi, but only at individual concentrations greater than 100 mg/L. The results have substantiated the multifunctional biological response of the Nutrinaft and Frucol products.

Further experiments were carried out for the identification of fungistatic active moieties in the Nutrinaft and Frucol products and the real surface dosage on the leaves and fruits. Thus, in any intermediary product the fungistatic effects are associated only with colloidal sulphur, as well as with potassium overbasic naphthenate and oleate. Only the last two are bearing multifunctional nutritive, growth enhancing and fungistatic biological activities. Mixing the intermediaries is an issue of formulating workable foliar fluids with dominant nutritive or fungicide tasks. These entire products perpetuate their biological functions in saline and variable pH solutions. Also, the experimental data do not complain with the theory of alkalinity sustaining antifungal properties, as happened in the case of inorganic fungicides like alkaline hydrogen carbonates or copper hydroxide.

REFERENCES

1. V. Chitu, E. Chitu, A. Hororoi, A. Perianu, F.C. Marin, A. Hororoi, M. Calogrea and L. Filipescu, "A new class of multifunctional foliar nutritive products", in: Scietifical Papers, University of Agronomic Sciences and Veterinary Medicine, Bucharest, B – XLVI, CD, 2003, pp.154-157;
2. C. Cristea, M. Calogrea, M. Murgea, V. Chitu and L. Filipescu, "Nutrinaft products and their fungicide effects", in: Analele Universitatii din Craiova, vol. IX (XLV), 2004, pp.103-109;
3. V. Chitu, E. Chitu, A. Hororoi, M. Calogrea, M. Murgea and L. Filipescu, "Researches concerning Nutrinaft products effects on apple production and fruit quality", in: Analele Universitatii din Craiova, vol. IX (XLV), 2004, pp.123-129;
4. M., Cirjaliu-Murcea, A.D., Ionita, E., Chitu and L. Filipescu, "Emulsified nutritive fluids. Overbasicity and hydrolysis control", in: Ann. Univ. Craiova, Romania 12, 2007. pp. 183-189;
5. C., Cristea, M., Cirjaliu-Murcea, A.D., Ionita, V., Chitu and L., Filipescu „Advanced Foliar Nutritive Fluids. II. Biological Response”, Annales of the University of Craiova, Vol XII (XLVIII), 2007, pp. pp.177-183;
6. C. Cristea, G. Campeanu, AD, Ionita and L., Filipescu, "The antifungal properties of the Frucol preparates," in: Romanian, Biotechnol Lett 12, 2007, pp3505–3512;
7. C. Cristea, G. Campeanu, AD, Ionita, L., Filipescu and M., Stefan, "The biological responses to the advanced foliar fluidic nutrients applications", in: Romanian Biotechnological Letters, vol 13(1), 2008, pp. 3583-3589;
8. C., Cristea, G., Campeanu, M., Cirjaliu-Murcea and L., Filipescu, „The fungidic effects of the Nutrinaft preparates, Romanian Biotechnological Letters:, 13, 2008, pp. 3589-3595;
9. E., Chitu, A.D., Ionita, M., Cirjaliu-Murcea, V., Chitu and L., Filipescu, "Evaluation of foliar nutritive fluids effect on apple photosystem II efficiency using chlorophyll fluorescence", in: Bulletin of USAMV Cluj-Napoca, Horticulture, 66, 2009, pp. 1843-5254;
10. C., Gaidau, E., Stepan, D., Taloi, M., Niculescu and L., Filipescu, "Additives and advanced biofertilizers obtained from leather industry by-products", in: Rev. de Chimie (Bucuresti), 60, 2009, pp. 501-507;
11. V., Chitu, E., Chitu, S., Nicolae, A.D., Ionita, M., Cirjaliu-Murcea and L., Filipescu, "Relationships between shelf life, health and quality of apple fruit", in: Acta Hort. (ISHS) 825, 2009, pp. 539-546;
12. V., Chitu, E., Chitu, F.C., Marin, A.D., Ionita, M. Cirjaliu-Murcea and Filipescu, L. "Effects of foliar ecological products application on apple growth, yield and quality", in: Acta Hort. (ISHS) 868, 2010 pp. 409-416;
13. L., Filipescu A.D., Ionita, M., Cirjaliu-Murcea, "Process for preparing two component nutritive fluids with multiple biologic action", in: RO 126401, 2011;
14. V., Chitu, E., Chitu, F.C., Marin and M., Pirlea, "Studies of Foliar Applied Clay Effects on the Apple Fruit Quality", in: Acta Hort. (ISHS) 884, 2010, pp. 441-447;
15. V., Chitu, V., Lacatus, C., Gaidau, A.D., Ionita and L., Filipescu, "Emulsified foliar nutritive fluid effects on the germination rates and leaves nitrate accumulation in plants", in: Revista de Chimie, 61, 2010, pp. 1025-1032;
16. M., Cirjaliu-Murcea, A.D., Ionita, P., Iordach and, L., Filipescu, "Emulsified Nutritive Fluids and Their Properties Control", in: Revista de Chimie (Bucharest) 62, 2011, pp.1-12;

17. C., Corobea M., Cîrjaliu Murgea A., Ioniță M., Calogrea and L., Filipescu, "Nanoparticulate sulphur in ecological fungicides", in: Proc. of 14th Romanian International Conference on Chemistry and Chemical Engineering, București, 3, 2005, 210-216;
18. J.H., Jorgensen, J.D., Turnidge and J.A., Washington, "Antibacterial susceptibility tests: dilution and disk diffusion methods". In: Murray PR, Pfaller MA, Tenover, FC, Baron, EJ, Yolkner, RH, ed. Manual of clinical microbiology, 7th ed.. Washington, DC: ASM Press, 1999, pp.1526-1543;
19. J.M., Andrews, "Determination of minimum inhibitory concentrations", J. Antimicrob. Chemotherapy, 48 (Suppl. S1), 2001, pp. 5-16;
20. H.J., Benson, Benson's Microbiological Application Laboratory Manual, 8th Edition, The McGraw-Hill Companies, 2001, pp. 51-57;
21. E., Lahoz, R., Contillo, F., Porrone, M., Avigliano and P., Iovien, "Efficacy of rue extract, sodium bicarbonate and fungicides at reduced rates to control of powdery mildew", Il Tabacco, 9, 2001, pp. 57-66;
22. R. K., Horst, S.O., Kawamoto, and L.L., Porter, "Effect of sodium bicarbonate and oils on the control of powdery mildew and black spot of roses", Plant Dis. 76, 1992, pp. 247-251.