The fungidic effects of the Nutrinaft preparates

CAMELIA CRISTEA*, GHEORGHE CAMPEANU, MARINA MURGEA*, LAURENTIU FILIPESCU***
** Faculty of Industrial Chemistry, University POLITEHNICA of Bucharest*
***Faculty of Biotechnology, Bd. Marasti No. 59, 71331, Bucharest, Romania*

Abstract

Nutrinaft class products are foliar applied nutritive emulsions with multiple biological functions. Their hydrolyzed entities are carried as micelles through plasma cell membranes accomplishing nutrients absorption and biostimulant feeding. Additionally, the organic film borne at the leaves surface acts as a contact fungicide. The subject of this paper concerns the fungicide properties of three products from Nutrinaft class.

Keywords: foliar fluids, fertilizers, biostimulants, fungicide

Introduction

Foliar nutritive products from Nutrinaft class are concentrated emulsions containing: macronutrients (nitrogen, phosphorus and potassium), mezonutrients (calcium, magnesium and sulphur), micronutrients (boron, molybdenum, zinc, copper, iron and manganese), biostimulants (naphthenic acids as ammonium, potassium, calcium, magnesium, zinc, copper, iron and manganese salts) and inorganic fungicides (mainly potassium bicarbonate and copper naphthenates) [1].

These fluids are not foliar fertilizers, biostimulants or growth control substances. Their modeled properties match all the required properties of foliar bioactive products providing vegetative growth, fruit yielding and quality, as well as protection against fungi and other fruit diseases, but the foliar nutrition mechanism involved when they are used as diluted hydrolyzing fluids is completely changed due to non-ionic transport of active entities through plasma cell membranes by overbasic naphthenates micelles [2]. Nutrinaft products are commercial brands of composite materials pioneering the field of plant nutritive fluids with multiple biological functions, formulated with a particular concern to environment friendliness [3].

Four brands of nutritive fluids with sequential application have been manufactured at laboratory and pilot scale and tested over a period of three years in experimental fields for vegetables and fruits production: Nutrinaft A (applied from February through April or all over the vegetative period in mixture with the other brands), Nutrinaft B (applied from May through June) Nutrinaft C (applied from July through September) and Nutrinaft D - FRUCOL (applied in September) [4-6]. The products Nutrinaft A, Nutrinaft B, Nutrinaft C and Nutrinaft D, whose compositions encompass all together the twelve nutritive elements are compatible after dilution, and, by mixing, give birth to complex nutritive fluids including NPK fertilizers, mezo and micronutrients, as well as biostimulants and fungicides. From this point of view, Nutrinaft products open a new market for two or three packs composite fluids in the field of foliar nutrition.

Results concerning contribution of this class of products as foliar fertilizers and growth enhancers in vegetable and fruit production have been previously published [2-5]. This paper deals with another feature of Nutrinaft products – their fungicide activity. This investigation purpose is to learn more about the particular products themselves and to develop a proper application technology.

Materials and Methods

A. Sample collecting. Samples of the organic materials (shots, leaves and fruits) were randomly collected from the experimental fields into two steps: first, one week before Nutrinaft products have been applied, and second after 2-3 days since products were applied. The selected samples were quickly transported into laboratory and assay samples were selected by visual scanning, holding back only the parts of plant which exhibit a potential infection. All the assay samples were introduced into deionized and sterilized water for isolation.

B. Inoculation. Samples of 1 ml were taken out from isolation media and spread over highly selective Czapec-Dox growth media, and respectively differential Sabouraud media freshly prepared on Petri disks. All disks were incubated for at least 10 days at 25°C.

C. Identification and specimen confirmation of fungi and yeasts. On the well-grown inoculated assay samples, typical species of fungi and yeasts were identified by macroscopic and microscopic observations and subsequently by comparison with the published literature [7]. Some inconclusive assay samples were checked for confirmation following specific biochemical tests [8-10]. Representative pictures were taken to illustrate the effect of Nutrinaft products.

D. Experimental development. All the experiments were carried out on the allotted surfaces in the orchards where Idared and Jonathan apple varieties are grown. The experimental lots were organized along three lines: first for blind assay tests (V3), second for the best commercial foliar product on the market (Megafol) (V1) and third for the sequential applied Nutrinaft products (V2). The results concern only the year 2004, which is the third in row since the experiments have been initiated. For each Nutrinaft product biological assay samples were collected before and after application. Each time the microaeroflora was examined. Only the results collected from Idared and Jonathan lots were presented in this paper, due to their continuous and constant bulk production during the entire experiment.

Results and Discussion

A. Nutrinaft products application and their action mechanism

The master core of the foliar nutritive fluids is made up by hydrolyzed over basic naphthenates of all the cations used as nutrients. Emulsified naphthenates were the physical support on which the typical foliar products properties have been grafted by changes in composition and constituent ratios.

Figure 1 describes the liquid-liquid phase equilibrium in a pseudo-ternary system Solvent – Naphthenic acids – Metal nitrate. The surface of different phase fields on diagram largely depends on the solvent composition, and the solvent contribution may be adjusted according to the particular formulation of any Nutrinaft product.

Figure 1 shows that concentrated emulsions are stable. When they are diluted, two liquid phases are separated, and when the dilution is very high, the two previously separated phases are reciprocally dissolving generating a stable emulsion or suspension.

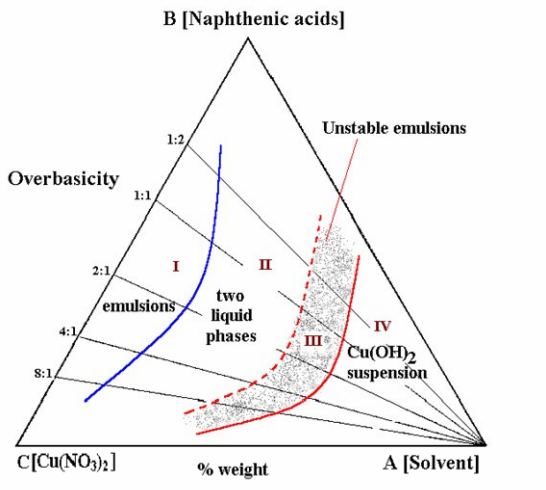


Figure 1. The hypothetical phase diagram of pseudothernary system
Solvent – Naphthenic acid – Metal nitrate

In fact, these emulsions and suspensions are the spread fluids on the leaf surface. When the solvent is evaporating, due to the specific properties of overbasic naphthenates, again two liquid phases are generated. The organic phase, which is a very dense and sticky one, spreads on the leaf surface and gives birth to a thin and adherent film. The water phase, which has a higher vapor pressure, evaporates leaving on the leaf surface a chemically reactive mixture. Thus, all soluble salts bearing nutritive elements are embedded in the organic film. At the same time, because the fluids have pH close to 10.0, during the water evaporation the precipitated film is quickly carbonated.

This process is accompanied by a significant drop in pH. Furthermore, the absorption of CO_2 results in hydrolysis of the overbasic naphthenates and the organic film breaks out in a discontinuous micelle structure.

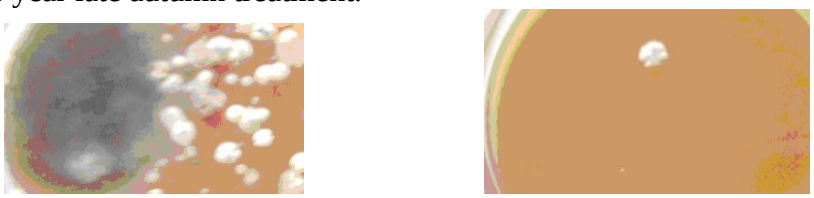
The micelles have a core of metallic carbonates/bicarbonates or hydroxides/ carbonates covered by a shell of naphthenic acids. Because the micelle film is soluble at a pH higher than 8.0, this mechanism of action excluded over dosages and let the leaf to take as much of the nutrients according to its capacity to generate alkaline metabolites. Due to its composition, the organic film liberates slowly and discontinuously, gradually, all the nutritive, biostimulant and fungicide entities.

B. Products fungicide capabilities

B.1. Nutrinaft A product. Because the first treatment with Nutrinaft A was made through March, before blooming, only shot assay samples were collected. Therefore, the results concern only the fungi development on branches and shots before bud bursting.

B.1.1. Experiments before application. For both Idared and Jonathan varieties fungi reached a maximum development after 96 hours incubation of the assay samples. On the Idared samples the dominant colonies of fungi were *Penicillium spp*., *Aspergillius spp*. and dimorph fungi. On Jonathan samples *Penicillium spp*., *Aspergillius spp*. *Alternaria spp*. and dimorph fungi made up dominant colonies. Figures 2 and 3 show the heavy fungi load on blind assays and poor fungi development on V2 assay samples, which may be considered as an evidence

for Nutrinaft A remnant antifungal action due to its application both in previous years and during previous year late autumn treatment.



Blind assay V2

Figure 2. Idared before treatment

Blind assay V2

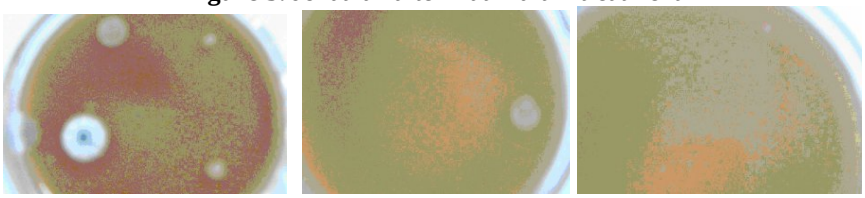
Figure 3. Jonathan before treatment

Blind assay V1 V2

Figure 4. Idared after Nutrinaft A treatment

Blind assay V1 V2

Figure 5. Jonathan after Nutrinaft A treatment



Blind assay V1 V2

B.1.2. Experiments after application. After the Nutrinaft A treatment, the blind assays collected for both apple varieties showed the maximum fungi loads after 10 days. The dominant species are *Botrytis spp*., *Aspergillius spp*. and lees for Idared samples, and, respectively, *Alternaria spp*. and *Aspergillius spp*. for Jonathan samples (figures 4 and 5). Lower fungi density on samples V2 collected from Idared apple experimental lot reveals the fungicide capacity of the Nutrinaft A product. One treatment seems to be conclusive for *Botrytis spp*. eradication, but inconclusive for other fungi removal. Two or more treatments may improve the product fungicide effects. Less infection was noticed on Jonathan apple experimental lot. Only blind assays exhibit fungi growth and multiplication. Clear grown assay samples collected from treated lots proved an undoubtedly evidence of the fungicide action of Nutrinaft A product.

Unfortunately, microaeroflora on experimental lots is dominated by *Botrytis spp.*, *Aspergillius spp*. and *Rodotorula spp*. (Jonathan), *Penicillium spp*., *Rodotorula spp*., *Botrytis spp*., *Fusarium spp*. and *Monillia spp*. (Idared) (figure 7). This may explain continuous infection produced by air circulation over the entire orchard, which, on one hand may impair all the experimental data, and on the other hand suggests a change in the antifungal treatments planning, as well as in the entire orchard management. As far as *Rodotorula spp*., *Fusarium spp*. and *Monillia spp*. did not appear on the inoculated assay samples, it seems that these fungi are not representative for the early vegetative stages.

B.2. Nutrinaft B and Nutrinaft C. In both experiments assay sample were processed according to the same procedure as in Nutrinaft A experiments. For Nutrinaft B experiment, assay samples were collected from leaves and fruits, but for Nutrinaft C, experiment assay samples were collected only from fruits. The experimental data are presented in table 1.

Table 1. Identified fungal forms after Nutrinaft B and Nutrinaft C treatments

Nutrinaft product	Idared	Jonathan
Nutrinaft B - leaves	**V3**: *Alternaria spp*., Dimorph fungi, Lees **V1**: Dimorph fungi, Lees, *Alternaria spp.* **V2**: Dimorph fungi, Lees	**V3**: Missing **V1**: Dimorph fungi, Lees **V2**: *Penicillium spp*., *Alternaria spp*., Dimorph fungi, Lees
Nutrinaft B – fruits	**V3**: Dimorph fungi, Lees **V1**: Missing **V2**: Dimorph fungi, Lees	**V3**: Dimorph fungi, Lees **V1**: Dimorph fungi, Lees **V2**: Missing
Nutrinaft C – fruits	**V3**: *Fusarium spp*., Dimorph fungi, Lees **V1**: *Alternaria spp*., Dimorph fungi, Lees **V2**: *Alternaria spp*., Dimorph fungi, Lees	**V3**: *Alternaria spp*., *Acremonium spp*., Dimorph fungi, Lees **V1**: Penicillium spp., Fusarium spp., Dimorph fungi, Lees **V2**: Botrytis spp., Penicillium spp., Dimorph fungi, Lees

Both products have no significant effect on dimorph fungi and lees, which could not be removed completely. Even if after the products treatments most of degenerated dimorph fungi are predominantly non-sporulating forms, the growth and multiplication do not cease.

Some fungi protection may be ascertained to Nutrinaft B on each apple variety, but Nutrinaft C seems to be missing any antifungal capacity. Repeated treatments with Nutrinaft B may improve product fungicide capacity but under highly charged microaeroflora the final results may be less satisfactory. Because Nutrinaft A exhibits certain fungicide properties, the mixing of this product with Nutrinaft B and, respectively, Nutrinaft C after convenient dilution may be seen as a sound solution for a significant increase in fungal activity of the entire class of Nutrinaft products.

Conclusions

1. There were disclosed some features of a new class of foliar nutritive fluids with multiple biological functions, whose core components are the overbasic metallic naphthenates;

2. Four sequential commercial products were designed in order to apply better the convenient dosages according to the vegetation stages;
3. All products fungicide capacities have been tested. Only Nutrinaft A use involve a specific fungicide capability which may be improved by dosage raise and a meaningful choice of the treatments number. Nutrinaft B exhibits poor fungicide properties promoting the degeneration of fungi to non - sporulated forms;
4. By mixing Nutrinaf A with each of the others products, fungicide capabilities may be extended over the periods the Nutrinaft B and Nutrinaft C are successfully applied to improve production and quality. Additionally, a new product – Nutrinaft D – was structurally designed to be mixed with other Nutrinaft products for a better control of their fungicide properties.

References

1. Petcu, F., Tabara, O., Filipescu, L. “Procedeu de fabricare a fluidelor nutritive cu actiune secventiala continand macronutrienti, mezonutrienti, micronutrienti si biostimulatori”, Dosar OSIM A/00130/2003;
2. Chitu Viorica, Chitu E., Perianu Adina, Marin F.C., Hororoi Anisoara, Calogrea Mihaela, Filipescu L. “A new class of multifunctional foliar nutritive products”, Lucrari stiintifice U.S.A.M.V.B., Seria B, Vol. XLVI, 2003, p.214;
3. Filipescu L. “Designing ecological products for agricultural uses” invited lecture University Venice, 2004
4. Calogrea M., Chitu V., Chitu E., Filipescu L. “A new class of multifunctional foliar nutritive products”, Proc. of 13th Romanian International Conference on Chemistry and Chemical Engineering, Bucuresti, 2003;
5. Marin C.F., Chitu V., Calogrea M., Filipescu L., Hororoi A. “Preliminary results regarding multifunctional foliar nutritive products use in walnut orchards”, Cercetari Stiintifice seria a VIII-a, Universitatea de Stiinte Agricole si Medicina Veterinara a Banatului, Ed. Agroprint Timisoara 2004;
6. Pascu Mihaela, Filipescu L. “New copper compounds with multiple biological functions” Proc. of 12th Romanian International Conference on Chemistry and Chemical Engineering, Bucuresti, 2001;
7. Konemon E.W., Allen S.D., Dowel V.R., Sommers H.M.”Color Atlas and Textbook of Diagnostic Microbiology” Lippicott Co., Philadelphia Toronto, 1979;
8. Barzoi D. “Microbiologia produselor alimentare de origine animala”, Editura Ceres, Bucuresti,1985;
9. Onet Emil, Constantinescu Virginia, Diagnosticul de laborator in medicina veterinara, Editura Ceres, Bucuresti, 1978;
10. Constantinescu Ovidiu “Metode si tehnici in micologie”, Editura Ceres, Bucuresti, 1974.