available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/indcrop

Review

Lignin and polyphenols as allelochemicals

V.I. Popa [a,*], Mariana Dumitru [a], Irina Volf [a], N. Anghel [b]

[a] "Gh. Asachi" Technical University, Faculty of Industrial Chemistry, Bvd. Mangeron no. 71, Iasi 700050, Romania
[b] "P. Poni" Institute of Macromolecular Chemistry, Gr. Ghica Voda no. 41 A, Iasi 700487, Romania

ARTICLE INFO

Keywords:
Polyphenols
Lignin
Allelopathy
Lignosulphonates

ABSTRACT

By the microbiological action, lignin from vegetal wastes is transformed at soil level in organic prebiotic products with physiological activity on plants development. On the other hand, some micromolecular compounds resulted from plant wastes decomposition, along with polyphenols coming from extraction of plant residues could play a role of allelochemicals. The understanding of the allelochemical action mechanisms allow us to use these compounds to enhance crop production and develop a more sustainable agriculture, including weed and pest control through crop rotations, residue management and a variety of approaches in biocontrol. Other goals are to adopt allelochemicals as herbicides, pesticides and growth stimulants, modify crop genomes to manipulate allelochemicals production and better elucidate chemical communications between the components of ecosystem. The result obtained in the utilization of lignins and polyphenols as allelochemicals are presented in this review.

Contents

1. Introduction 144
2. Area descriptions 146
 2.1. Materials and methods 146
  2.1.1. Biotests 147
3. Results and discussions 148
4. Conclusions 149
 References 149

1. Introduction

The phenomenon of allelopathy encompasses all types of chemical interactions among plants and microorganisms. Several hundred different organic compounds (allelochemicals) released from plants and microbes are known to affect the growth or aspects of function of the receiving species.

The allelochemicals influence patterns in vegetation communities, plant succession, seed preservation, germination

* *Corresponding author*. Tel.: +40 232 271311; fax: +40 232 271311.
E-mail address: vipopa@ch.tuiasi.ro (V.I. Popa).
0926-6690/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2007.07.019

of fungal spores, the nitrogen cycle mutuality association, crop productivity and plant defense. Allelopathy is tightly coupled with competition for resources and stress from disease, temperature extremes, moisture deficit and herbicides. Such stresses often increase allelochemical production and accentuate their action. Allelophatic inhibition typically results from a combination of allelochemicals which interfere with several physiological processes in the receiving plant or microorganism (Einhelling, 1995).

Other than the ante-ecological study of specific species, there are persistent challenges in allelopathy to determine the mechanism of action of compounds, isolate new compounds, evaluate environmental interactions and understand activity in the soil. New frontiers will focus on ways to capitalize on allelopathy to enhance crop production and develop a more sustainable agriculture, including weed and pest control through crop rotations, residue management and a variety of approaches in biocontrol.

Other goals are to adopt allelochemicals as herbicides, pesticides and growth stimulants, modify crop genomes to manipulate allelochemical production and better elucidate chemical communications that generate associations between microorganisms and higher plants.

Often, the immediate source of a compound involved in allelopathy is obscure. For example, microorganisms may alter compounds released from higher plants before another higher plant contacts the altered substance. Similarly, it is very difficult to establish the source when a compound of any origin is contacted through the soil medium. A further complication is that the same compound is likely to have multiple roles, affecting different kinds of recipient plant.

Higher plants regularly release organic compounds by volatilization from their surfaces and through leaf leachates and root exudates.

Likewise, even during their limited life span, the various microorganisms do not retain all that they produce. Toxic release is the mechanisms of action of many fungal pathogens, antibiotic zones improve the success of certain bacteria, and microbial signals lead to some associations.

The source of allelochemicals may be either the crop, weeds or microorganisms of the decomposition processes. Eventually, the chemical constituents of all organisms are released to the environment through the process of decomposition. Some are volatile compounds that permeate the air environment of the soil as well as having some solubility in the aqueous phase (Aldrich, 1987).

Allelochemicals transfer from one higher plant to another in a terrestrial community can be either through volatiles, aqueous leachates or various exudates. Volatiles may move through the atmosphere from a donor plant to a receiving species; alternatively, these compounds are adsorbed on soil particles and solubilized in the soil solution. Water-soluble allelochemicals leached from shoot tissue into the soil matrix and exudates from roots are a regular occurrence.

Hence, spatial movements of allelochemicals can be of some distance and they often differ that the soil acts as allelochemical pool. Roots of a receiving plant take up allelochemicals from the soil solution or lipid-soluble compounds adsorbed on soil particles can partition directly into root tissue. Plant residues decomposing in the soil will result in localized regions of higher allelochemical concentrations and the impact of allelochemicals in the soil on a receiving plant often depends on the chance encounters of the root system with such region.

With a few exceptions, the allelopathic agents reported from higher plants are secondary compounds that arise from either the acetate or shikimate pathway, or their chemical skeleton come from a combination of these two origins (Fig. 1).

The kind of plant material added to soil markedly affected the kinds and members of microorganisms; soil invertase, amylase and cellulase activity also decreased with progression of old-field succession.

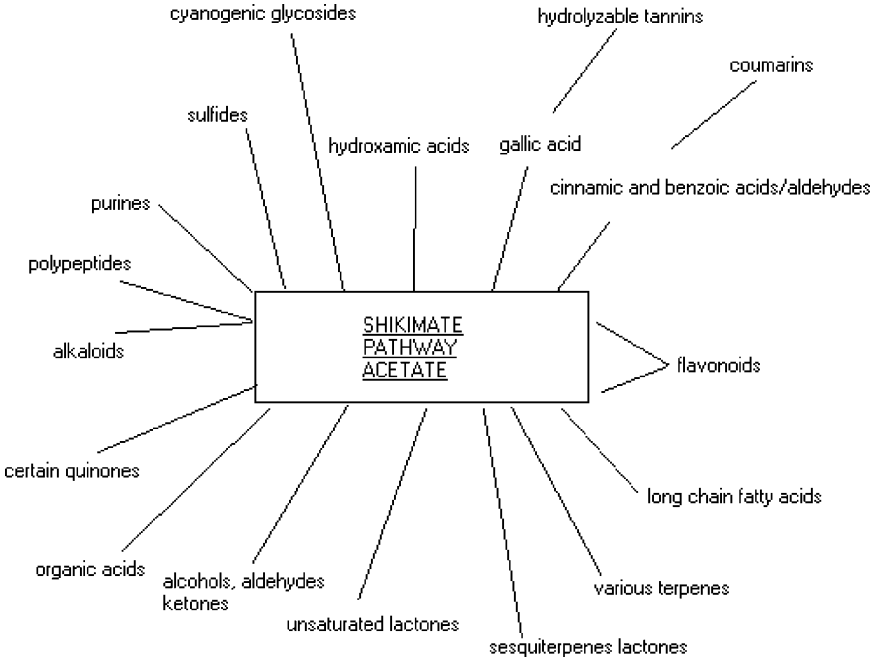

Fig. 1 – Some of diversity of allelochemicals implicated in allelopathy.

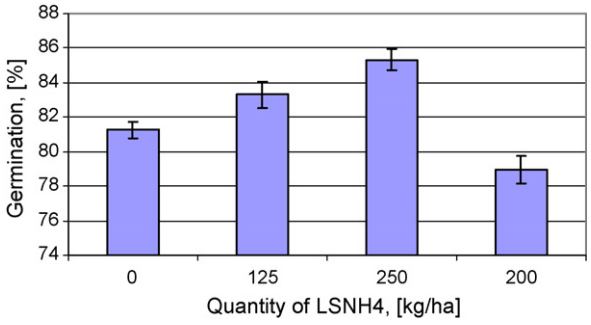

Fig. 2 – Influence of $LSNH_4$ addition upon the germination capacity of bean seeds.

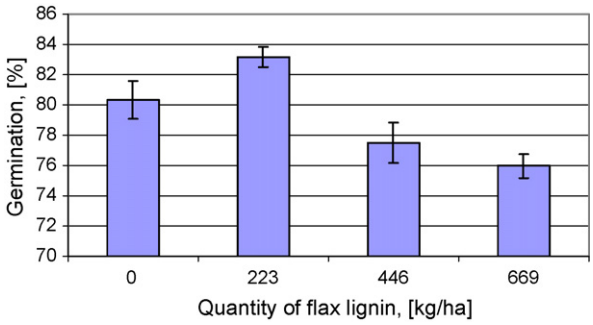

Fig. 3 – Influence of flax lignin addition upon the germination capacity of bean seeds.

Effects on the receiving crop plants and other weeds include reduction in chlorophyll, water uptake, nutrient uptake and legume nodulation. Several sesquiterpene lactones, phenolic acids and organic acids have been identified as the responsible agents.

In our studies different lignins and polyphenols extracted from phytomass sources have been used in model experiments to follow their actions as allelochemicals. Data concerning the role of polyphenols has been presented somewhere (Popa et al., 1998, 2000; Dumitru et al., 2002, 2003). The extraction of polyphenol compounds from different kinds of phytomass in order to recovery and to increase the economic value of these by-products is an actual research theme approached by several research groups (Basile et al., 2000; Taylor and Grotewold, 2005; Schijlen et al., 2004).

In this paper the results obtained in the study of lignosulfonates and flax lignin are discussed as well as polyphenolic solutions extracted from *Vitis* wood.

2. Area descriptions

2.1. *Materials and methods*

Alkaline flax lignins: lignin from flax delignification (type PF 30-35 Flax Soda Pulping), from "Granit-Lignin", Switzerland.

Ammonium lignosulphonates ($LSNH_4$): residual bisulphitic solution with ammonia as base from Romanian sulfite pulp mill, SC "Celohart" SA Zarnesti, with an initial concentration of 25.65 g/L organic matter.

Polyphenolic extract from Vitis species: in view to obtain alkaline extract, old wood and stems of *Vitis* sp., ground and screened, have been used. Aqueous solution of natrium hydroxide has been utilized as extraction agents. The optimal working condition (concentration of NaOH solution 1.5%, liquid/solid ratio 10 cm^3/g, duration 3 h, temperature 90 °C) has been established by factorial programming of experiments. The obtained vegetal extract was a light brown opalescent liquid, with relative density 1.1024, dried substance 6.06%, ashes

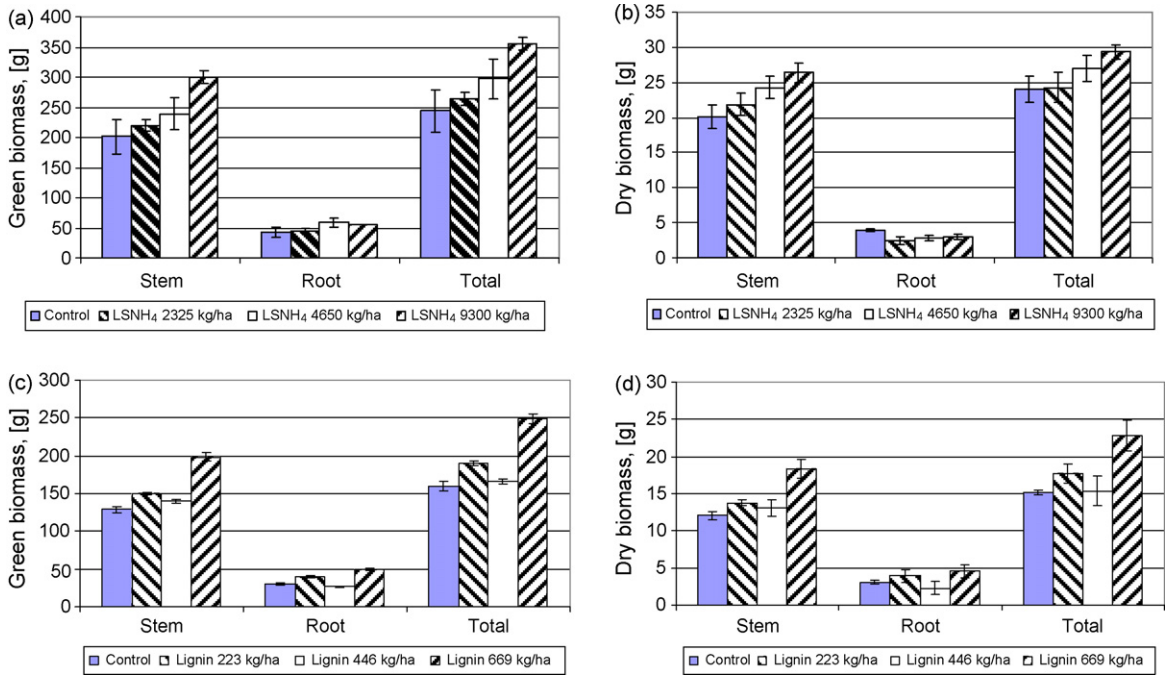

Fig. 4 – Variation of green biomass with the $LSNH_4$ addition (a) and flax lignin addition (c); variation of dry biomass with $LSNH_4$ addition and (b) flax lignin addition (d).

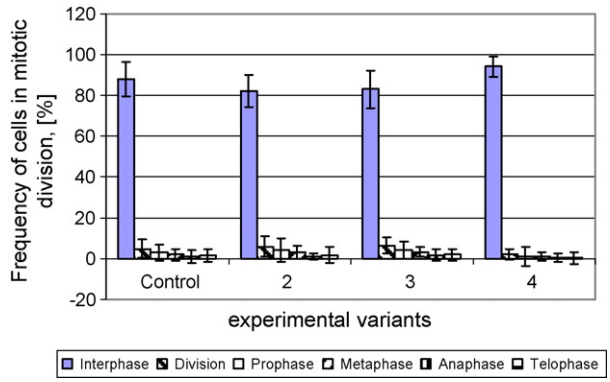

Fig. 5 – The variation of mitotic index in the cells of radicular meristems of *P. vulgaris* under the effect of lignin treatment: 2–223 kg lignin/ha, 3–446 kg lignin/ha, 4–669 kg lignin/ha.

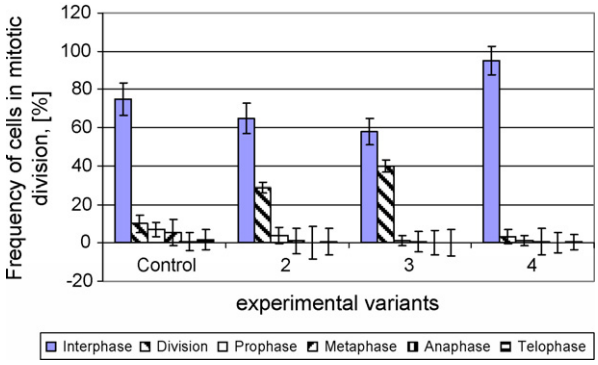

Fig. 6 – The variation of mitotic index in the cells of radicular meristems of *P. vulgaris* under the effect of $LSNH_4$ treatment: (2) 2325 kg $LSNH_4$/ha, (3) 4650 kg $LSNH_4$/ha, (4) 9300 kg $LSNH_4$/ha.

2.057%, organic substance 4%, total nitrogen 28 mg/L, reduced sugars 0.1 g/L, and total polyphenols 2.7% (Volf and Popa, 2004). Generic name of total polyphenols includes phenolic acids and acetophenones, hydroxycinnamic acids, coumarins, stilbens, flavonoids, lignans, catechine and oligomers and condensed tannins, etc. For using the obtained extract in this experiment the pH was set to 7.

Selected bean seeds (*Phaseolus vulgaris*): type "*Magna*" from "Agricultural Research Station Podu Iloaiei"—Iasi, Romania.

2.1.1. *Biotests*

Plant cultivation: in vegetable pots for 40 days, using as culture media quartz sand with 400, ..., 800 μm granulation, and in field for 3 months, both with different addition of biologically active compounds.

Germination: ten bean seeds per Petri dish on filter paper, at 25 °C for 4 days. Beans seeds were soaked in 10 mL water for reference, and 10 mL of various concentrations of biologically active compounds (ammonium lignosulphonates: 0.25 g/L (125 kg/ha), 0.5 g/L (250 kg/ha), 1.0 g/L (500 kg/ha); flax lignin: 0.25 g/L, 0.5 g/L, and 1.0 g/L). Tests were performed in triplicate with 10 Petri dishes per concentration.

Laboratory phase: concerned in investigation of germination capacity and mitotic division on the *P. vulgaris* beans embrionary roots. Beans from every variant were germinated in Petri dishes, on distilled water, at 25 °C, in dark place. Roots of 1, ..., 1.5 cm were fixed with Battaglia fixator prior to be submitted to cytological investigations. It were also investigated the mutagenic effects of lignin on biological materials, performing cytogenetic study of chromosomal aberrations in mitosis anatelophase of embrionary roots of beans. Investigation on the mitotic index at the level of radix moistens of *P. vulgaris*: colouring agent—Carbol-Fuchsine (CARR), fixating agent—alcohol–acetic acid 3/1; microscope Nikon E600 Eclipse, 25×, object lens 100×.

Grafting biotest: the polyphenolic extract has been tested, at different levels of concentration, in a biotest of induction of new plant tissue (callus) in grafting process for obtaining planting material. For this purpose, the treatment of grafting point with aqueous polyphenolic solutions in following range of concentration was completed: 0.1, 0.25, 0.5, 1.0 g/L (organic material). The observations were made comparatively with reference (water) and with a commercial callusogenesis regulator.

The experiment was made on Busuioaca de Bohotin sort grafted on Berlandieri × Ripparia SO4-4 root stock under industrial condition (at least 100 vines for each concentration, 3 year). After the treatment, vines were paraffined on the grafting point, without introducing anticryptogamic substances in mixture. After the forcing operation, there were immediately made observations regarding the presence of circular callus in grafting zone.

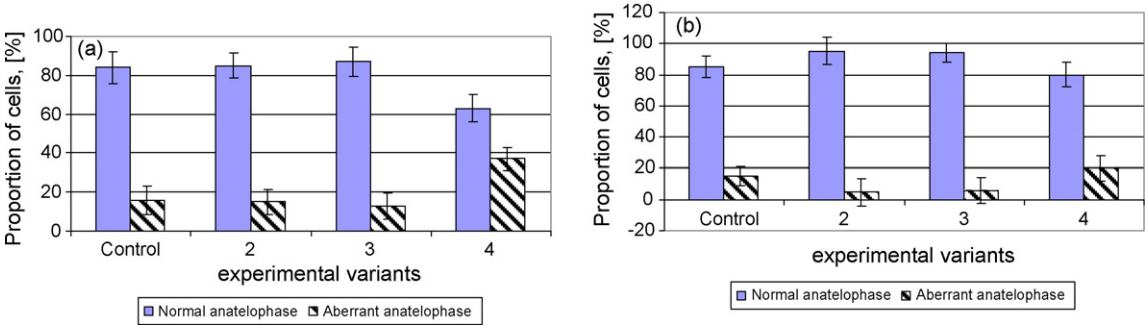

Fig. 7 – The proportion of aberrant anatelophases in the cells of radicular meristems of *P. vulgaris* under the effect of flax lignin (a) and $LSNH_4$ (b) treatment.

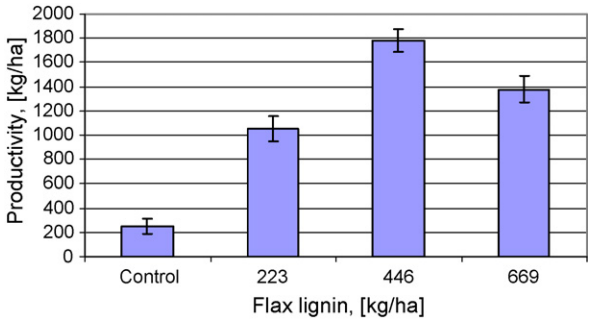

Fig. 8 – Productivity of *P. vulgaris* seeds as a function of flax lignin add-on.

3. Results and discussions

Fig. 2 shows that ammonium lignosulphonate positively influences the germination process, till a certain concentration; at higher concentration the germination process is inhibited. Fig. 3 shows that in the case of flax lignin utilization in the germination process, the later is lower than in case of lignosulphonates use. The situation can be correlated with the difference of solubility of lignin products which influences the accessibility of the substances to the biological material.

Ammonium lignosulphonate positively influences the quantity of green and dry biomass accumulated in stems, while in the roots the accumulation takes place randomly (Fig. 4).

In the case of flax lignin, similar to the case of ammonium lignosulphonates, a positive influence is noticed on the quantity of green and dry biomass accumulated in stems (Fig. 4).

It is expected that the lignin products suffer transformations under the action of the microorganisms developed in the soil and these degradation products play a role in the development of plants. To this purpose, a first parameter investigated was the mitotic index, its value proving information on the influence of lignin and ammonium lignosulphonate, respectively on the mitotic dividing at the level of radix moistens of *P. vulgaris*.

As shown in the graphs below, both flax lignin (Fig. 5) and ammonium lignosulphonate (Fig. 6) induce the increase of the number of dividing cells, as compared with the control sample. This aspect is characteristic for the first two concentrations used because for lignin and also for ammonium lignosulphonate, the third higher concentration induce a slight decrease of the frequency of dividing cells, correlated with an increase of the frequency of cells in the interphase.

Over a certain concentration level, both products inhibit the mitotic dividing process, which leads to a lower growing rhythm and, implicitly, to a lower productivity.

Another parameter that must be investigated in the experiments concerning the biostimulating effects of a compound is represented by the frequency of the normal/aberrant anatelophases in the cells of radicular meristems.

Luckily, the results obtained indicate a behavior that slightly induces chromosomal aberrations, therefore a low mutagenic capacity for both lignin and for LSNH4 (Fig. 7). In the case of the first two concentrations, for lignin and for LSNH4, the level of aberrant anatelophases is lower than in the control sample, where the aberrations appear naturally, in a certain proportion, due to mutagenic factors present in the environment.

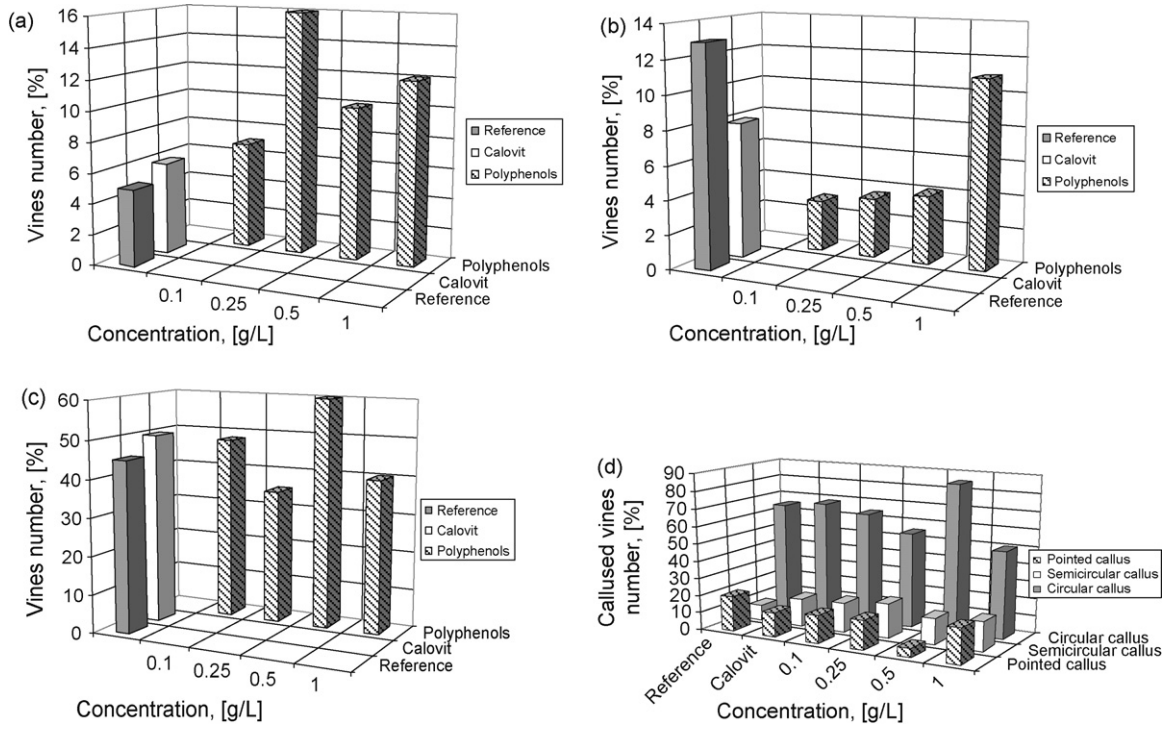

Fig. 9 – Influence of additons of polyphenols from *Vitis* wood on the formation of (a) semicircular callus and shoots; (b) formation of pointed callus and shoots; (c) formation of circular callus and shoots and (d) formation of different callus types.

In this context, we consider that, beside the biostimulating effect of flax lignin and LSNH4, a protective effect appears, especially for LSNH4, effect proven by the decrease of the level of aberrant anatelophase in the vegetal material treated with specific concentrations of these compounds.

Fig. 8 presents the productivity of *P. vulgaris* seeds as a dependence of flax lignin addition in a field experiment (Fig. 9) (Dumitru et al., 2003).

In the case of callus formation process, the experiments confirm the biostimulating effect of polyphenolic extract used. It can be noticed that the 0.5 g/L concentration domain for which the number of vines with circular callus and sucker is even higher than that treated with commercial biostimulator (Calovit) (Tudose and Popa, 2000).

Taking into account only the formation of different kinds of callus without considering the development of sucker, the biostimulative influence of polyphenolic extract is more evident, the number of vines with circular callus being in this case about with 20% higher than reference (Popa et al., 2000).

4. Conclusions

From presented data we can say that there are sufficient arguments to state that polyphenolic and ligninic products and their derivatives can act as allelochemicals. The action of these compounds depends on their nature, composition, concentration, procedure and degree of modification.

The investigations presented allow to appreciate that both lignin and $LSNH_4$ have a biostimulating effect on mitotic division, in the radicular meristems of *P. vulgaris*. It is possible that this effect is induced, in the case of lignin, as a result of the improvement of micro media conditions at plants' roots level, correlated with the beneficient influence of lignin on the flora present in soil.

This effect is doubled by the protective character of these compounds' presence, especially of $LSNH_4$, materialized in the diminishing of the aberrant anatelophases frequency at a lower level than the one of the control sample.

Exciting goals are to adopt allelochemicals as herbicides, pesticides and growth stimulants, modify crop genomes to manipulate allelochemicals production and better elucidate chemical communications between the components of ecosystem.

In summary, the pervasive involvement of plant-produced chemicals in plant–plant and plant–microorganism interactions provides many challenging frontiers. This science has the potential to contribute greatly to agricultural production and stability.

REFERENCES

Aldrich, R.J., 1987. Interference between crops and weeds. In: Waller, G.R. (Ed.), Allelochemicals: Role in Agriculture and Forestry, ACS Symposium Series No. 330. American Chemical Society, Washington, DC, pp. 300–311.

Basile, A., Sorbo, S., Giordano, S., Ricciardi, L., Ferrara, S., Montesano, D., Castaldo Cobianchi, R., Vuotto, M.L., Ferrara, L., 2000. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. Fitoterapia 71 (Suppl. 1), S110–S116.

Dumitru, M., Obreja, I., Campeanu, C.S., Campeanu, M.M., Popa, V.I., 2002. Lignin enriched arid-soil effects on biochemical markers in *Phaseolus vulgaris*. In: Annual Science Symposium on Actual and Perspectives in Horticulture, Iasi, pp. 413–416.

Dumitru, M., Popa, V.I., Obreja, I., Campeanu, M.M., 2003. The influence of some lignin products on the metabolic processes of plants. Bull. Polytechnic Inst. Jassy Tome XLIX (LIII), 109–118.

Einhelling, F.A., 1995. Allelopathy: current status and future goals. In: Inderjit, K.M.M., Einhelling, F.A. (Eds.), Allelopathy: Organisms, Processes and Applications, ACS Symposium Series No. 582. American Chemical Society, Washington, DC, pp. 1–7.

Popa, V.I., Anghel, N.C., Popa, M., Cotzur, D., 1998. On the biological properties of lignin products and polyphenols. In: Proceedings of the Fourth International Forum of International Lignin Institute on Lignin from Pulping of Agricultural Plants, Present and Future, Lausanne, pp. 1–15.

Popa, V.I., Tudose, I., Anghel, N.C., 2000. Influence of polyphenols and lignin products an the biosynthesis processes. In: Proceedings of the Fifth International Forum of International Lignin Institute, Bordeaux, pp. 1–12.

Schijlen, E.G.W.M., Ric de Vos, C.H., van Tunen, A.J., Bovy, A.G., 2004. Modification of flavonoid biosynthesis in crop plants. Phytochemistry 65 (19), 2631–2648.

Taylor, L.P., Grotewold, E., 2005. Flavonoids as developmental regulators. Curr. Opin. Plant Biol. 8 (3), 317–323.

Tudose, I., Popa, V.I., 2000. Plants regulators from stems of *Vitis vinifera*. advances in lignocellulosics chemistry towards high quality processes and products. In: Proceedings of the Sixth European Workshop on Lignocellulosics and Pulp, Bordeaux, France, pp. 633–636.

Volf, I., Popa, V.I., 2004. Rev. Chim. 55 (9), 33–39.