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The Effect of pH on Production of Plant Growth Regulators by Mycorrhizal Fungi

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Abstract

Plant growth regulators are considered to be of importance in mycorrhizae formation and function. Seven isolates of ectomycorrhiza forming fungi were used. The fungi were grown in Lamb's medium at pH 4.0, 5.8 and 7.0. All the fungi studied produced auxin-like substances (ALS) in tryptophan containing media. More auxins were detected at pH 5.8 and 7.0 than at pH 4.0. Among the ALS, IAA was mainly produced. Only one fungal isolate produced ALS in medium of pH 5.8-without tryptophan. Gibberellin-like substances (GLS) were produced by 4 isolates. The production of these substances was inhibited at pH 7.0. In the post culture media of *Cenococcum graniforme* and *Hebeloma mesophaeum*, GA_3-acetate was detected by gas chromatography. Cytokinin-like substances (CLS) were produced by 6 fungal isolates grown at pH 5.8 and pH 7.0. No correlation was found between production of biomass and CLS synthesis. Among the CLS produced, 2iP riboside and zeatin riboside were detected. We realize that the results obtained *in vitro* cannot be directly transferred to the situation in nature. However only studies carried out *in vitro* can indicate the potential physiological capabilities of microorganisms studied.

Keywords: Ectomycorrhizal fungi, pH, auxin-, cytokinin-, gibberellin-like substances

Abbreviations: ALS: auxin-like substances; GLS: gibberellin-like substances, CLS: cytokinin-like substances; IAA: 3-indoleacetic acid; IAN: β-indolylacetonitrile; GA_3: gibberellic acid; GA_5: gibberellin A_5 methyl ester; GA_7: gibberellin A_7 methyl ester; Z: zeatin; ZR: zeatin riboside; 2ip: 6γ,γ-(dimethylallyloamino) purine; 2iPR: 2iP riboside

1. Introduction

The importance of the hormonal factor in formation and functioning of mycorrhiza has been stressed by many workers (Slankis, 1973; Meyer, 1974; Barea, 1986; Gogala, 1991). It is known that plant growth regulators produced by mycorrhizal fungi affect root morphology and metabolic changes in the host plant (Slankis, 1973). Auxins and cytokinins are of special interest (Gogala, 1991). Production of these plant growth regulators by mycorrhizal fungi in different media of pH between 5.4 to 6.0 is well documented (Kampert and Strzelczyk, 1978; Rudawska, 1982; Strzelczyk and Pokojska-Burdziej, 1984; Ho, 1987a,b; Gay et al., 1989; Kampert and Strzelczyk, 1989).

On the other hand, the plant growth hormones contained in roots and root exudates influence growth and metabolic activity of mycorrhizal fungi (Gogala, 1991). The role of plant growth substances liberated by microorganisms accompanying mycorrhizas cannot be neglected in mycorrhizal studies (Kampert and Strzelczyk, 1984; Strzelczyk and Pokojska-Burdziej, 1984; Strzelczyk et al., 1987). Plant hormones among other metabolites are considered to be responsible for the balance between the partners of mycorrhizal associations (Ho, 1987a). A shift in this balance may alter or disturb these relationships.

The formation of mycorrhiza depends upon pH (Theodorou and Bowen, 1969; Metzler and Oberwinkler, 1987). pH affects not only fungal growth (Hung and Trappe, 1983) and growth of the root, but also the physiological activity of both partners. Little is known about the effect of pH on the production of plant growth regulators by mycorrhizal fungi. Therefore this study was undertaken.

2. Materials and Methods

Fungi

Seven mycorrhizal fungi were used (Table 1).

Culture conditions

The fungi were grown in static cultures for 21 days at 26°C in Lamb's medium (1974) of different pH: 4.0, 5.8 and 7.0. Two discs (1 cm in diameter) of the fungi grown for 7–14 days on Potato Dextrose Agar (Difco) were

Table 1. Isolates of mycorrhizal fungi

Fungal species	No. of isolate	Year of isolation	Isolated	Source of	Mycorrhiza formation test	
					ECM	EEM
Suillus bovinus (L. ex Fr.) O. Kuntze	1941	1970	sporocarp	Notec Forest, Poland	+	–
Hebeloma crustuliniforme (Bull. ex Fr.) Quel.	5397	1984	sporocarp	Innsbruck, Austria	not tested	
Hebeloma mesophaeum (Pers. ex Fr.) Quel.	3037	1971	sporocarp	Notec Forests, Poland	+	–
Hebeloma crustuliniforme (Bull. ex Fr.) Quel	5392	1974	sporocarp	Nancy, France	+	–
Ectoendomycorrhizal fungus Mrg X	4	1984	ectoendomycorrhiza of *Pinus sylvestris*	Sekocin, Poland	–	+
Cenococcum graniforme (Sow) Ferd. et Winge	3543	1971	ectomycorrhiza of *Abies alba*	Saint Cross Mountains, Poland	+	–
Pisolithus tinctorius (Pers.) Coker et Couch	5335	1974	ectomycorrhizae of *Pinus taeda*	USA	+	–

ECM = ectomycorrhiza
EEM = ectoendomycorrhiza

placed in 200 ml of medium in 1000 ml Erlenmeyer flasks. Auxin production was studied in L-tryptophan supplemented media (0.2 g/l). Studies on the production of these substances in tryptophan-free medium (pH 5.8) were also performed. For detection of ALS and GLS, experiments were set up in triplicate; for detection of CLS, five flasks were inoculated and the post culture liquids were combined and extracted.

Extraction, purification of the extract, chromatography and bioassays of auxin-, gibberellin-, and cytokinin-like substances were performed as described earlier (Strzelczyk and Pokojska-Burdziej, 1984; Kampert and Strzelczyk, 1984). For more accurate identification of the substances showing auxin-, gibberellin- and cytokinin-like activity in the biotests, selected extracts of fungal isolates were studied by means of gas chromatography (Gas Chromatograph "Chromatron" model GCHF 18.3–4). The following plant growth regulators were used as standards: IAA, IAN, GA_3, GA_3 3-acetate, GA_5, GA_7, ZR, Z, 2iPR, 2iP (Sigma). Detection of auxins was performed in samples methylated with diazomethane. Gibberellins were studied in samples additionally silylated with BSA/bis/Trimethylsilyl/acetamide according to the method of Jolliffe et al. (1979). The glass column, 200 cm × 0.4 cm, was packed with 5% SE-30 on Gas-Chrom Q, 100–120 mesh. Column temperatures were set up at 180°C (for auxins) or 250°C(for gibberellins), injection and detector temperatures at 280–290°C. Flame Ionization Detector (FID) was used. The carrier gas was N_2, at a flow rate of 40 cm^3 min^{-1}. Detection of cytokinins in extracts of *Suillus bovinus* and *Hebeloma crustuliniforme* 5392 by means of gas chromatography was performed according to the method given earlier (Strzelczyk and Kampert, 1987).

3. Results

Auxin-like substances

In tryptophan-free medium (pH 5.8) ALS were detected only in the post culture filtrates of *Pisolithus tinctorius* in amount 18.5 μg equivalent IAA/g of dry mass. Gas chromatography analysis revealed the presence of IAA (Fig. 1). All the fungi studied produced different amounts of ALS in tryptophan containing media. The highest activity was exhibited by *Pisolithus tinctorius* (Table 2, Fig. 3). The pH of the media, in most cases, affected the amount of ALS produced. As a rule, more auxins were detected at pH 5.8 or 7.0 than at pH 4.0 (Table 2, Fig. 2).

On the basis of the results obtained by the *Avena* coleoptile test and gas chromatography, it is assumed that the main active substance among ALS was IAA (Figs. 2–3). This auxin (localized on paper chromatograms at R_f 0.3–0.5)

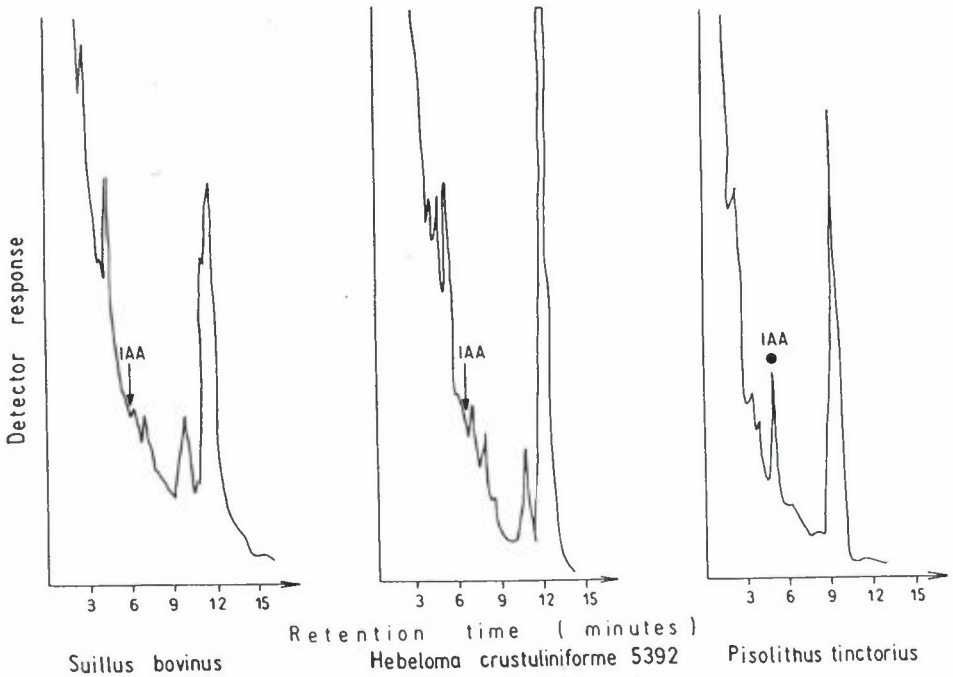

Figure 1. Gas chromatography of methylated derivatives of ALS in selected isolates of mycorrhizal fungi grown in medium of pH 5.8 without tryptophan.

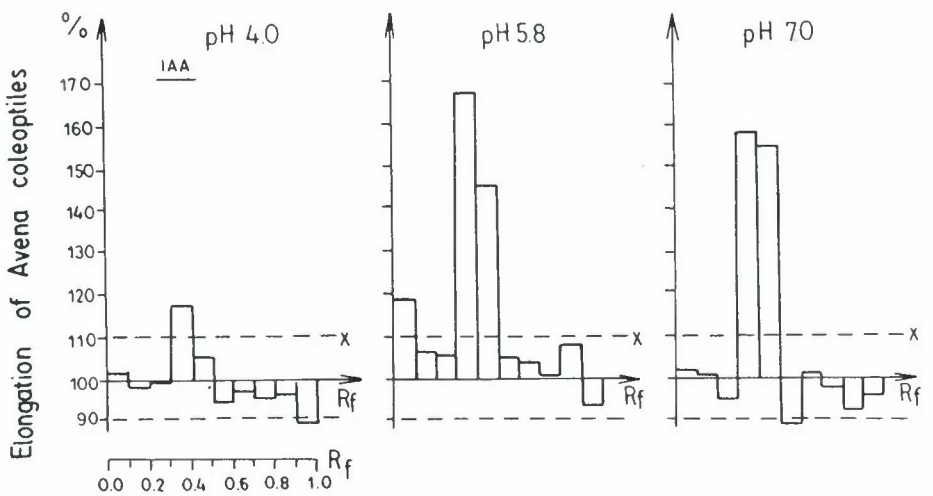

Figure 2. Paper chromatography of ALS produced by *Suillus bovinus* in media of different pH. Explanation to Figs. 2& 4: the portions above the line x indicate significant differences at P=0.05.

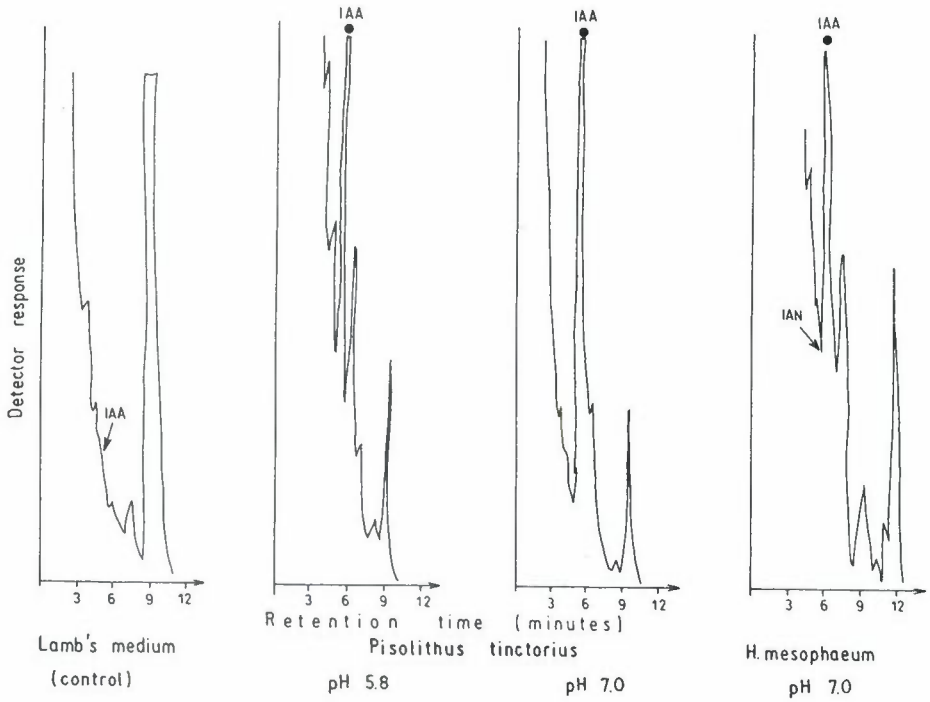

Figure 3. Gas chromatography of methylated derivatives of ALS produced by *Pisolithus tinctorius* and *Hebeloma mesophaeum* in media of different pH.

was produced by all the fungi studied (Table 2). Additionally, less active zones in the biotest were found in the culture liquids of most fungi (at R_f 0.6–0.7; 0.7–0.8; 0.8–1.0). These substances were not identified. IAN was not detected in the extract of *Hebeloma mesophaeum* (Fig. 3).

Gibberellin-like substances

Four isolates produced substances stimulating lettuce hypocotyls in the biotest for gibberellins. Production of these substances was inhibited at pH 7.0 (Table 3). GLS were located on the chromatograms at different R_f values (Fig. 4). In the extracts of fungi showing GLS activity, GA_3 and GA_7 were not detected by means of gas chromatography. In the post culture liquids of *Cenococcum graniforme* and *Hebeloma mesophaeum*, GA_3 3-acetate was found. Revealing of GA_5 was not possible because retention time of this gibberellin and glucose (present in the media) was almost identical. Standards of other gibberellins were not available. Substances showing inhibitory effect on the

Table 2. Auxin-like substances production by mycorrhizal fungi in Lamb's medium of different pH

Fungi	pH 4.0		pH 5.8		pH 7.0	
	Amount of ALS expressed as IAA equivalents (means of three replicates)					
	μg/g of dry weight	R_f	μg/g of dry weight	R_f	μg/g of dry weight	R_f
Suillus bovinus	8.7	0.3–0.4	57.9	0.3–0.5	45.1	0.3–0.5
Hebeloma crustuliniforme 5397	no growth		82.7	0.3–0.5	162.4	0.2–0.5 0.8–0.9
Hebeloma mesophaeum	63.1	0.3–0.5	78.2	0.3–0.5 0.8–1.0	68.3	0.3–0.5 0.8–1.0
Hebeloma crustuliniforme 5392	11.6	0.4–0.5	51.9	0.3–0.5	33.8	0.3–0.5 0.6–0.7
Ectendomycorrhizal fungus Mrg X	trace	0.3–0.4 0.6–0.7	44.6	0.3–0.4	14.1	0.3–0.4 0.7–0.8
Cenococcum graniforme	0		6.3	0.4–0.5	191.9	0.2–0.5
Pisolithus tinctorius	119.1	0.2–0.5	108.1	0.3–0.5 0.9–1.0	259.8	0.2–0.5

Table 3. Gibberellin-like substances production by mycorrhizal fungi in Lamb's medium of different pH

Fungi	pH 4.0		pH 5.8		pH 7.0	
	Amount of GLS expressed as GA_3 equivalents (means of three replicates)					
	μg/g of dry weight	R_f	μg/g of dry weight	R_f	μg/g of dry weight	R_f
Suillus bovinus	trace	0.5–0.6	13.4	0.0–0.3	0	–
Hebeloma crustuliniforme 5397	no growth		0	–	0	– 0.8–0.9
Hebeloma mesophaeum	15.2	0.5–0.6	0	–	trace	0.1–0.2
Hebeloma crustuliniforme 5392	0	–	0	–	0	–
Ectendomycorrhizal fungus Mrg X	0	–	26.9	0.0–0.2	0	–
Cenococcum graniforme	91.3	0.0–0.1 0.2–0.4 0.5–0.6	5.3	0.2–0.3	0	–
Pisolithus tinctorius	not studied		0	–	0	–

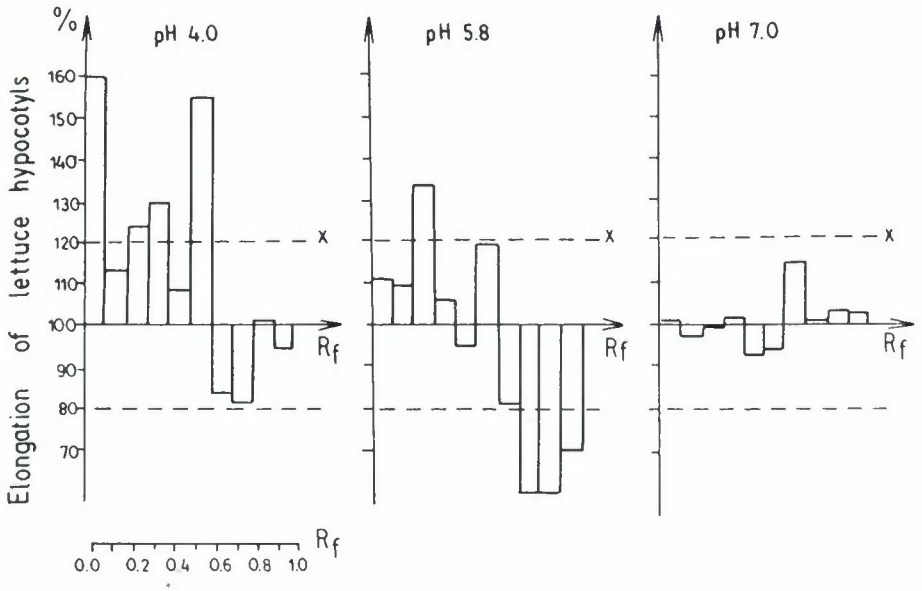

Figure 4. Thin layer chromatography of GLS produced by *Cenococcum graniforme* in media of different pH.

lettuce hypocotyl's growth were present in the post culture liquids of all fungi studies (Fig. 4).

Cytokinin-like substances

Six isolates produced CLS in media of pH 5.8 and 7.0 and four in those of pH 4.0 (Table 4). In medium of pH 5.8 the highest stimulation of soybean callus was caused by *Hebeloma crustuliniforme* 5392 (column fractions 16–40) and *Suillus bovinus*, which stimulated the soybean callus by all fractions. The substances detected by biotest corresponded to 2iPR, ZR and Z (Figs. 5–6).

The greatest amounts of CLS were produced by the ectendomycorrhizal fungus Mrg X and *Cenococcum graniforme* at pH 7.0 (Table 4). The amount of CLS produced varied between 0.016 to 0.05 μg equivalent kinetin/g of dry mass of mycelium grown at pH 4.0, between 0.018 to 0.084 at 5.8 and 0.005 to 0.157 at pH 7.0.

Gas chromatographic analysis did not confirm the results obtained by the soybean callus test. It is possible that among CLS substances, other than those used as standards (2iP, R2iP, RZ and zeatin) were produced.

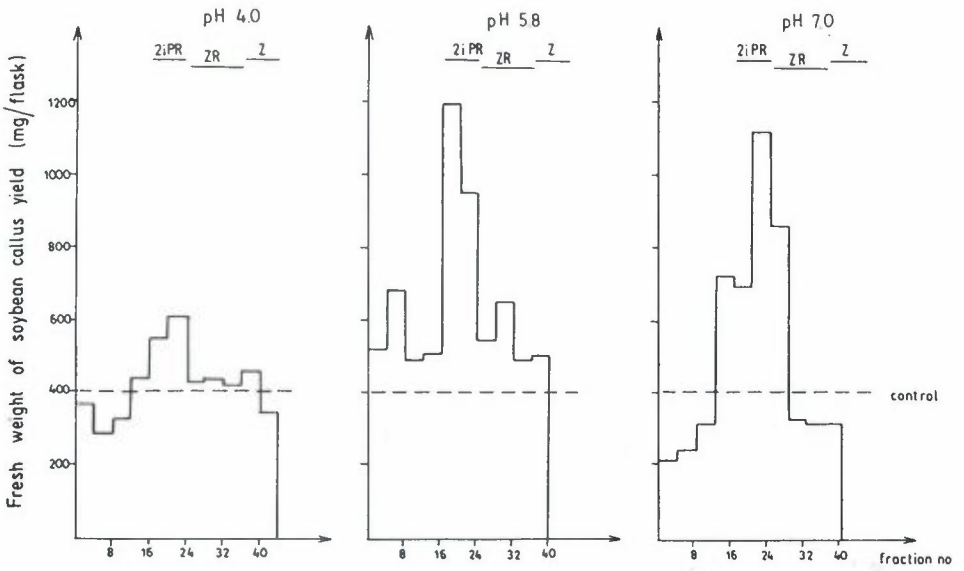

Figure 5. Chromatographic analysis of CLS produced by *Hebeloma crustuliniforme* 5392.

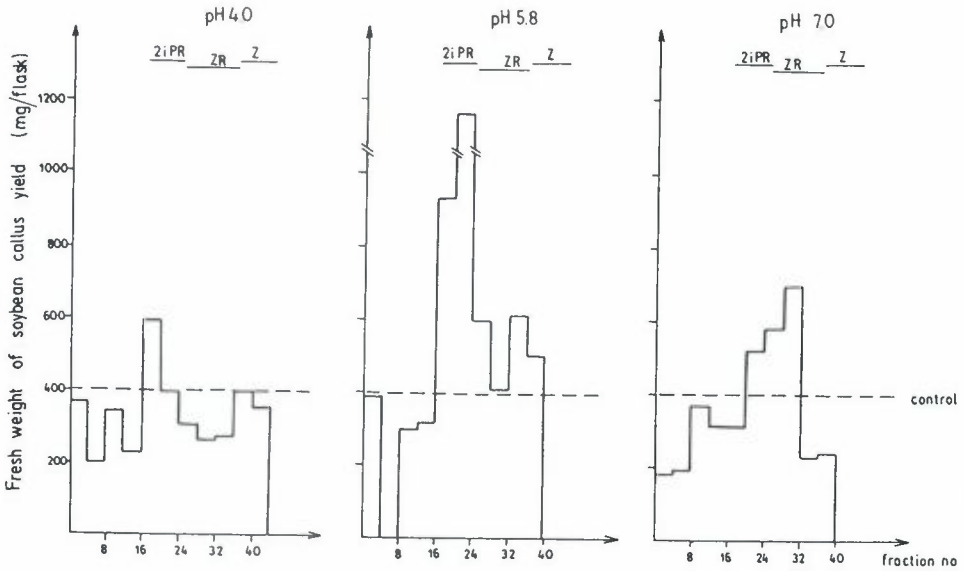

Figure 6. Chromatographic analysis of CLS produced by *Suillus bovinus*.

Table 4. Cytokinin-like substances production by mycorrhizal fungi in Lamb's medium of different pH

Fungi	pH 4.0			pH 5.8			pH 7.0		
	Amount of cytokinin-like substances expressed as kinetic equivalents								
	Biomass (g/l)	μg/g of dry weight	Fraction number	Biomass (g/l)	μg/g of dry weight	Fraction number	Biomass (g/l)	μg/g of dry weight	Fraction number
Suillus bovinus	1.022	0.049	12–40	1.1647	0.084	0–40	0.9399	0.055	12–28
Hebeloma crustuliniforme 5397	no growth			0.4894	0.026	12–20	0.4674	0.051	16–28
Hebeloma mesophaeum	0.2811	0.033	20–40	1.1402	0.019	20–32	1.5989	0.005	20–24
Ectendomycorrhizal fungus Mrg X	no growth			0.3922	0.052	8–12 & 20–28	0.4542	0.101	8–12 & 16–32
Cenococcum graniforme	0.371	0.05	16–24	0.821	0.018	8–12 & 24–28	0.4085	0.157	8–36
Pisolithus tinctorius	0.8465	0	–	1.4235	0	–	1.1845	0	–

4. Discussion

Among the plant growth regulators auxins and cytokinins are considered to be of importance for mycorrhizae formation (Gogala, 1991). Auxins are at least partially responsible for stunting and dichotomy of ectomycorrhizal roots and they influence carbohydrate transport in plants (Slankis, 1973). Cytokinins are important, not only because of their influence on growth and development of higher plants, but also on the mycorrhizal fungi (Gogala and Pohleven, 1976). Almost nothing is known about the role of gibberellins in mycorrhiza formation and functioning. However, the production of gibberellin-like substances by mycorrhizal fungi has been well documented (Strzelczyk, Sitek and Kowalski, 1975; Strzelczyk and Pokojska-Burdziej, 1984; Hanley and Greene, 1987; Ho, 1987a). Differences in the production of plant growth substances by mycorrhizal fungi as reported by Gay and Debaud (1987) and by Ho (1987a,b) could be the reason of different effects exerted by different strains of these fungi on growth of the host plant (Mikola, 1973).

Many chemical and physical factors may affect the production of plant growth substances by mycorrhizal fungi. Effects of organic substances which are available in the root zone are of special interest. It was shown that amino acids, sugars, organic acids and vitamins affect synthesis of auxins as well as cytokinins (Rudawska, 1982; Gay, 1986a; Strzelczyk and Kampert, 1987; Pokojska and Strzelczyk, 1988; Kampert and Strzelczyk, 1989; Strzelczyk and Pokojska, 1989). It also was found that production of plant hormones by the fungi was not growth-linked.

Mycorrhizal fungi in general prefer fairly acidic substrates for their growth. But optimal pH for mycorrhiza formation may not be at the same pH value as optimal for mycelial growth (Metzler and Oberwinkler, 1987).

In our studies more auxin- and cytokinin-like substances were produced by the fungi at pH 5.8 and 7.0 than at pH 4.0. An alkaline pH may enhance the production of auxins by microorganisms. It was found by Phelps and Sequeira (1968) that *Pseudomonas solanacearum* produced most auxins at pH 6–9. It was shown by Gay (1986b) that activity of the enzymes synthesizing IAA in *Hebeloma hiemale* was highest at alkaline pH. Rubery and Sheldrake (1974) and Raven (1975) postulated that alkaline pH can induce IAA release from cells whereas IAA uptake by higher plant cells is stimulated by acid exocellular pH. Contrary to the production of ALS and CLS, the production of GLS was greater at lower pH. The importance of gibberellins for mycorrhizal fungi and mycorrhiza formation is not known and deserves further study.

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