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Full Length Research Paper

Allelopathic potential of macrofungi on germinating maize (*Zea mays* L.) grain

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The effect of methanol extracts of 10 macrofungi was evaluated on grain germination of maize (*Zea mays* L.). Germination percentage, radicle and plumule length and the level of carbohydrates and fatty acids were measured. Fungal metabolites inhibited germination up to 90.96%, plumule (97.77%) and radicle (92.83%) development. Plumule and radicle lengths were stimulated 35.26 and 10.60% in some assays, respectively. The fungal metabolites decreased the glucose (97.60%), sucrose (90.34%), fructose (96.85%), maltose (95.64%), oleic acid (97.50%) and linoleic acid (98.25%) levels, whereas increasing levels of the glucose (165.14%), sucrose (166.53%), fructose (83.18%), maltose (124.73%), oleic acid (6975.00%) and linoleic acid (5233.33%) were detected in some assays. It is concluded that macrofungi metabolites have commonly inhibitory effects on physiological and morphological processes of germinating maize grain except for considerable increases in the some parameters investigated.

Key words: Allelopathy, carbohydrate, fatty acid, fungal metabolites, germination, maize.

INTRODUCTION

Metabolites of many fungi may have adverse or stimulatory effects on plants (Heisey et al., 1985; Rice, 1995) such as suppression of seed germination, malformations and retardation of seedling growth (Lynch and Clark, 1984). These metabolites seem to provide a promising herbicidal activity of several microfungi on weed plants (Nakajima et al., 1991; Kastanias and Chrysayi-Tokousbalides, 2000; Zeng et al., 2001; Idris et al., 2003; Zhi-Qi et al., 2005; Meriles et al, 2006). Several crude extracts of fungal species have showed to affect germination and growth of weed species (Phattanawasin et al., 2006). For example, extracts from *Lactarius hatsudake* inhibited root and shoot development of rape, radish and barnyardgrass and stimulated rice shoot height (Mo et al., 2005). The activity of metabolites from macrofungi may be also used together to synthetic biocides. The germination and development of weeds were suppressed by bioherbicidal and soil-born fungi at different inoculum concentrations alone and in combination with glyphosate (Molish, 1937; Boyette et al., 2008).

Allelopathy is a useful tool in sustainable agriculture (Rizvi et al., 1999). Most allelopathy research is focused on weed suppression (Narwal, 1994; Rice, 1995; Kastanias and Chrysayi-Tokousbalides, 2000; Kadioğlu and Yanar, 2004). Nevertheless, physiological effects and action mechanisms of allelopathic chemicals are sometimes not well understood. The aim of this work was to evaluate the effects of macrofungi extracts on physiology of grain germination and seedling growth using maize as a model system.

Although maize plant does not share the same living place with macrofungi tested, it was used as a model plant to understand the effects of alleochemicals in macrofungi on physiological and developmental processes in plants. Some allelochemicals in macrofungi tested may

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Abbreviations: HPLC, High performance liquid chromatography; **MF,** macrofungi; **MFE,** macrofungi extracts; **LA,** linoleic acid; **OA,** oleic acid; **ANOVA,** analysis of variance.

be useful as alternative to synthetic compounds used for producing more crops.

MATERIAL AND METHODS

Macrofungi Material

The following macrofungi were collected from Gümüşhane and Artvin provinces (Turkey): *Morchella vulgaris* (Pers.) Boud, *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys and Moncalvo, *Leucoagaricus leucothites* (Vittad.) M.M. Moser ex Bon, *Macrolepiota procera* (Scop.) Singer var. *procera*, *Pleurotus ostreatus* (Jacq.) P. Kumm, *Amanita muscaria* (L.) Lam. var. *muscaria*, *Volvariella gloiocephala* (DC.) Boekhout and Enderle, *Pholiota aurivella* (Batsch) Fr., *Suillus luteus* (L.) Roussel and *Polyporus squamosus* (Huds.) Fr. The identification of macrofungi was carried out according to related literature (Moser, 1983; Breitenbach and Kränzlin, 1984-2000; Buczacki, 1989; Jordan, 1995). The macrofungi specimens were preserved in VANF herbarium, Yuzuncu Yil University, Van, Turkey. The collected macrofungi were dried in laboratory according to mycological methods.

Preparation of the methanol extracts

Twenty grams of fruiting bodies of dried macrofungi were powdered in liquid nitrogen. Each powder was shaken with 200 ml of methanol for 24 h at room temperature in the dark. Then, the extract was filtered through Whatman filter paper (No.1) and methanol was removed under vacuum at 40°C using a rotary evaporator. The residues obtained were stored at -80°C until use.

Bioassay of macrofungi extracts on maize grain

Grains of *Zea mays* with uniform size were immersed in 2.5% (w/v) NaOCl for 3 min. After sterilization, grains were washed with sterile distilled water and dried with sterile filter paper. Grains were soaked in sterile distilled water for 1 h and 15 grains were germinated in Petri dishes on four layers of sterile Whatman No.1 filter paper. Concentration of macrofungi extracts was adjusted to 3.3, 5.0 and 10.0% (w/v) with deionized water. Then, 20 ml of each extract were added to each Petri dish containing 15 grains and allowed to germinate at 25°C in dark for 7 days. Seedlings were harvested with root lengths of 1.5 - 3.0 cm. Plumule and radicle length were measured and germination percentage was calculated and then they were maintained until analysis at -80°C. The macrofungi extracts were tested in triplicate for each parameter. Sterile distilled water was added to a Petri dish containing 15 grains as control. For biochemical and physiological analysis the germinated grains were used.

Determination of fatty acids

Quantitative determination of oleic and linoleic acids was performed according to the methods of Christie (1997) and Hamrouni et al. (2001). Two grams of sample were ground to powder in liquid nitrogen and homogenized in 20 ml of a chloroform : methanol mixture (2:1). Then, the mixture was diluted 1/5 by adding 4 ml of double distilled water. The mixture was separated in two phases. The upper layer was removed and the lower layer containing the fatty acids was evaporated under vacuum. The residue was dissolved by methanol and injected into HPLC equipped with a µBondapak C_{18} column (Waters, Hicrom Ltd. UK); Waters 6000 A pump (Waters, Hicrom Ltd. UK); Refractive Index detector (Waters 2414). Acetonitrile: water (78:22) was used as mobile phase at a flow rate of 1.5 ml/min. The absorption wavelength was chosen at 412 nm. The retention times for linoleic and oleic acid standards were 6.25 and 9.04 min, respectively.

Determination of free sugars

Free sugars were analyzed by the methods of Torije et al. (1998) and Karkacier et al. (2003). Five grams of sample were powdered in liquid nitrogen and 40 ml of methanol was added. The mixture was incubated on a magnetic stirrer at 65°C for 30 min. Then, it was centrifuged at 4°C, 1300 rpm for 40 min. The supernatant was transferred in a clean tube and made up to 50 ml with methanol. Then, methanol was removed in a rotary evaporator and the residue was dissolved in 25 ml of double distilled water. The extract was passed through Sep-Pak C_{18} cartridge and 2.5 ml of filtrate was mixed with 7.5 ml acetonitrile. Then, it was filtrated with 0.45 µm membrane filter and injected into HPLC equipped with Spherisorb 5 µm NH_2 column (250 × 4.6 mm; Waters, Ireland); Waters 6000 A pump (Waters, Hicrom Ltd. Uk); Refractive Index detector (Waters 2414). Acetonitrile : water (80:20) was used as the mobile phase at a 1.5 ml/min flow rate. Eluted compounds were detected at 412 nm. The retention times were determined for fructose (5.12 min), glucose (5.65 min), sucrose (7.87 min) and maltose (9.25 min).

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The statistical analyses were made using the Minitab 13 for windows packet program. Means and Standard deviations were calculated according to the standard methods for all parameters. One way ANOVA statistical test was used to determine the differences between means of the treatments and the control group accepting the significance level at $p < 0.05$. The statistically significant changes were indicated on the figures and table by asterisks.

RESULTS

Effect of macrofungi extracts on grain germination

Several macrofungi extracts significantly reduced percentage of grain germination. The highest inhibition of grain germination was observed for extracts of *V. gloiocephala* and *P. aurivella*, *M. vulgaris* and *M. procera* var. *procera* at concentration of 5% and S. *luteus* at 10% (Figure 1).

Effect of macrofungi extracts on seedling growth

The plumule growth of germinating maize was inhibited by some extracts and stimulated by others. Plumule growth was stimulated by extracts of *Pleurotus ostreatus* at 3.3 and 10%, *A. muscaria* var. *muscaria* at 10%, *P. squamosus* and *Volvaria gloicephala* at 5% and *L. leucothites* at 3.3% (Figure 2). The most suppressive effect on plumule length was determined for *V. gloiocephala and S. luteus* at 10% and *M. vulgaris* at 5%.

Macrofungi extracts inhibited radicle length of maize except for 10% of *A. muscaria* var. *muscaria* and 3.3% of *L. leucothites* where radicle length was higher than that of

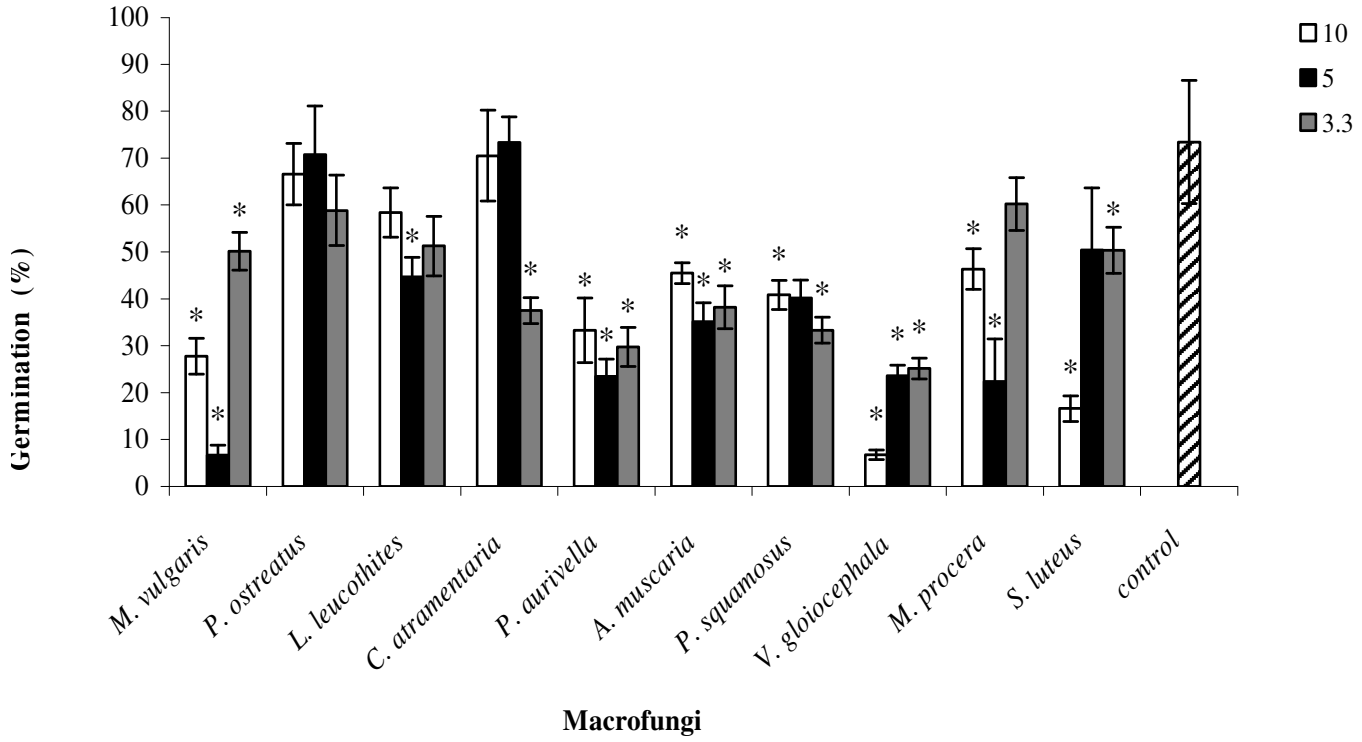

Figure 1. The effect of macrofungi extracts on germination of maize plant.

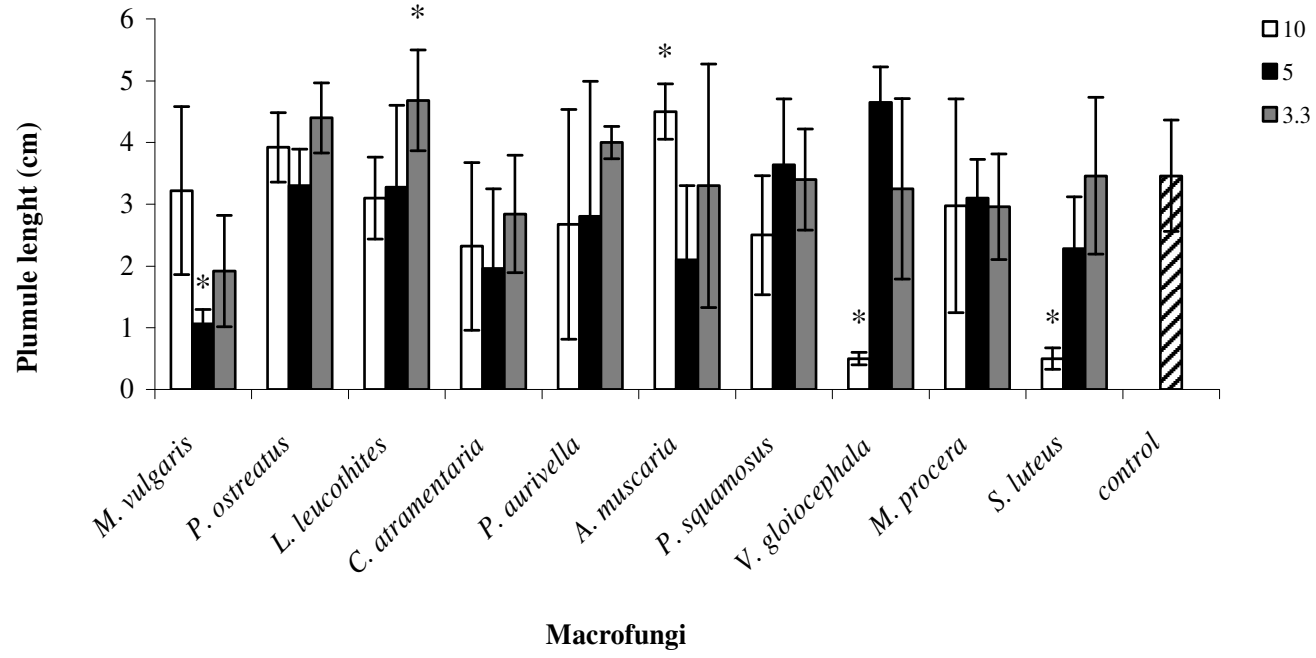

Figure 2. The effect of macrofungi extracts on plumule length of maize plant.

the control. The most suppressive effect of macrofungi extract on radicle development was determined in the applications of all *P. ostreatus*, 10% of *V. gloiocephala* and *S. luteus* and 5% of *M. vulgaris* extracts (Figure 3).

Physiological parameters

The decreased glucose levels were also increased with changing dilution amount of macrofungi extracts (MFE).

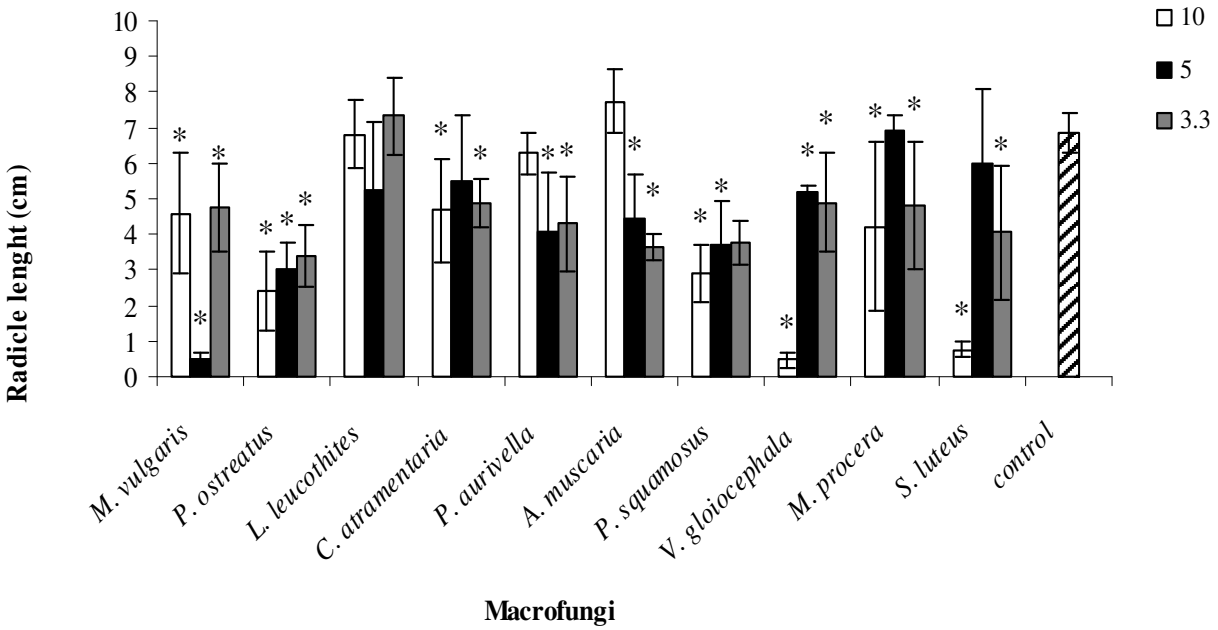

Figure 3. The effect of macrofungi extracts on radicle length of maize plant.

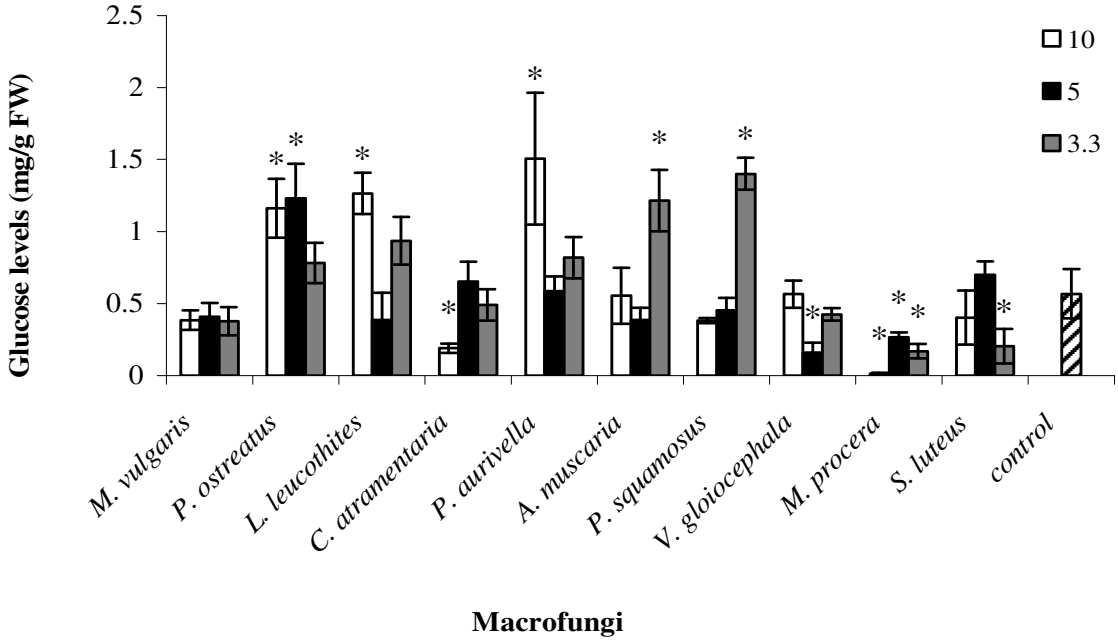

Figure 4. The effect of macrofungi extracts on glucose levels in maize plant.

All *P. ostreatus* and *P. aurivella* applications and 5% of *S. luteus* and *C. atramentaria*, 3.3% of *P. squamosus*, *A. muscaria* var. *muscaria* and 10 and 3.3% of *L. leucothites* extracts increased glucose levels whereas, all *M. procera*. var. *procera* and *M. vulgaris* extracts and 10% of *C. atramentaria*, 5% of *V. gloiocephala*, 10 and 3.3% of *S. luteus*, 5% of *L. leucothites*, *A. muscaria* var. *muscaria*, 10 and 5% of *P. squamosus* extracts decreased glucose levels (Figure 4). The lowest glucose level of germinating maize grains was determined in the applications of *M. procera* var. *procera* extract.

The 10% of *M. vulgaris*, *P. ostreatus*, *L. leucothides*, *P. aurivella* and *V. gloiocephala* and 3.3% of *V. gloicephala*, *P. squamosus* and *A. muscaria* var. *muscaria* and 5% of *P. aurivella* extracts increased the sucrose level compared to that of the control. The highest sucrose levels were

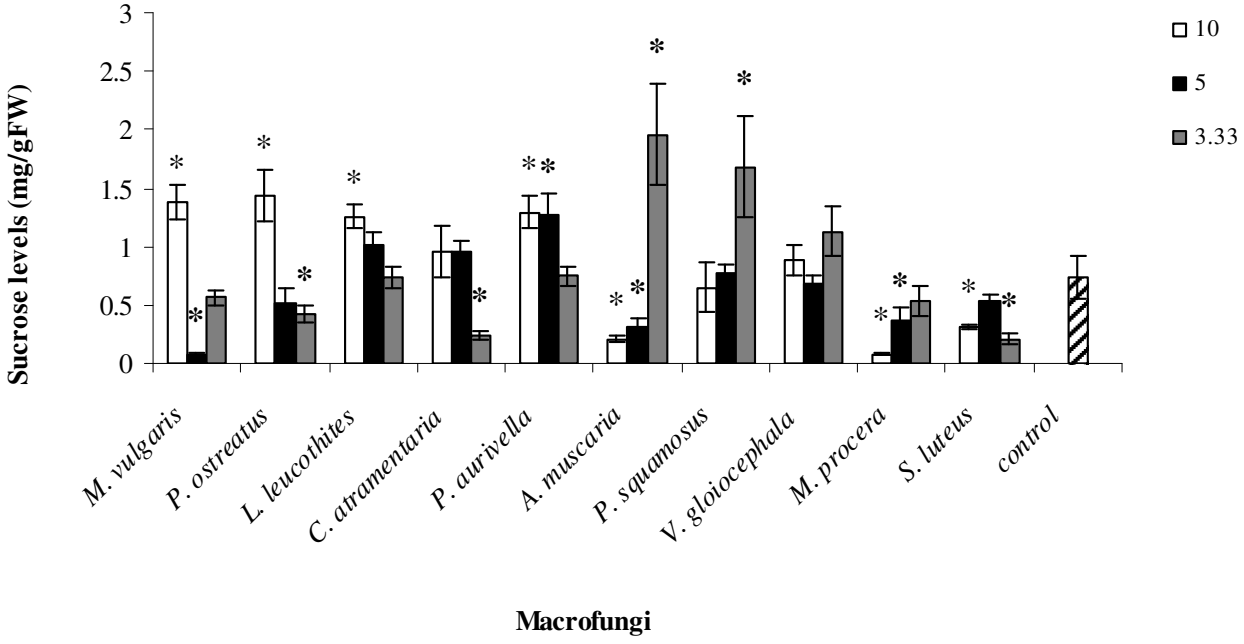

Figure 5. The effect of macrofungi extracts on sucrose levels in maize plant.

seen in the application of 3.3% of *A. muscaria* var. *muscaria* and *P. squamosus* extracts. *M. procera* var. *procera*, *S. luteus* extracts decreased sucrose levels. The lowest sucrose levels were determined in 5% of *M. vulgaris*, 5 and 3.3% of *P. ostreatus*, 3.3% of *C. atramentaria*, 10 and 5% of *A. muscaria* var. *muscaria* extract applications (Figure 5).

In general, fructose level was seriously inhibited by macrofungi extracts, whereas 10% of *P. squamosus* and 5% of *P. aurivella* and *M. procera* var. *procera* extracts increased fructose level compared to that of the control. The lowest fructose levels were determined in the application of 10% of *M. procera* var. *procera*, *P. aurivella* and *C. atramentaria*, 5 and 3.3% of *P. squamosus*, *C. atramentaria*, *M. vulgaris* and *S. luteus* extracts. The most inhibited results on fructose concentration were observed in *L. leucothites*, *P. ostreatus* and *V. gloiocephala* extract applications where any fructose amount was not detected by HPLC (Figure 6).

Maltose concentrations decreased in changing levels with different dilution of macrofungi extract applications except for all *P. squamosus*, 5% of *A. muscaria* var. *muscaria* and 3.3% of *P. aurivella*, *L. leucothites* extract where maltose levels were found to be higher than that of the control. The significantly lowest maltose levels were observed in the applications of *M. vulgaris* and *P. ostreatus* extracts, 10 and 3.3% of *S. luteus*, *V. gloiocephala*, 10 and 5% of *L. leucothites*, *C. atramentaria* extracts (Figure 7).

The MFE increased linoleic acid (LA) levels except for 5 and 3.3% of *M. procera* extracts. The highest LA levels were found in the applications of 10% *V. gloiocephala*, *P. squamosus*, *C. atramentaria*, *M. vulgaris*, 5 and 3.3% of *S. luteus*, *P. squamosus*, *A. muscaria* var. *muscaria, C. atramentaria* and *P. ostreatus* extracts (Table 1).

The oleic acid (OA) levels in general were decreased by MFE applications. All *M. vulgaris*, *A. muscaria*, *M. procera* var. *procera*, *S. luteus* and 10 and 3.3% of *P. squamosus*, 5 and 3.3% of *P. aurivella*, *V. gloiocephala* extract applications did not reveal any OA level in germinating maize grains. The OA levels were found to be higher in all *P. ostreatus*, *C. atramentaria* and 5% of *P. squamosus* extract applications than that of control (Table 1).

DISCUSSION

The extracts from macrofungi (MF) species assayed showed a significant inhibitory effect on grain germination and plumule and radicle growth. This inhibition may be due to the alteration of enzyme activity, which affects the mobilization of storage compounds during germination (Einhellig, 1995). The data are in accordance with Mo et al. (2005) findings indicating that extract of *L. hatsudake* inhibited the root development of plants. The most important effect of allelopathy was reported to be on seed germination (Rizvi et al., 1999). The increasing dilution rate of extracts is expected to cause decrease in the inhibition level of maize grain germination. The results presented (Figure 1) are in contrast to the previous findings in the literature suggesting that higher concentration of allelopatic chemicals may cause higher level of phytotoxicity in plant germination (Kadioğlu and Yanar,

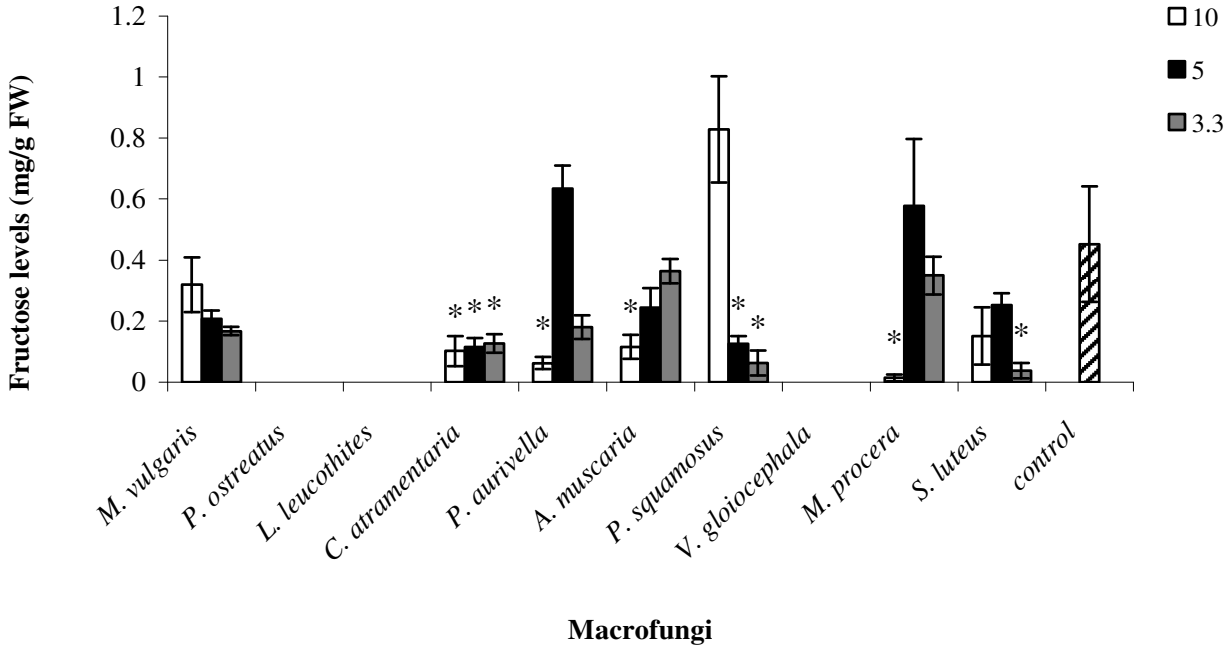

Figure 6. The effect of macrofungi extracts on fructose levels in maize plant.

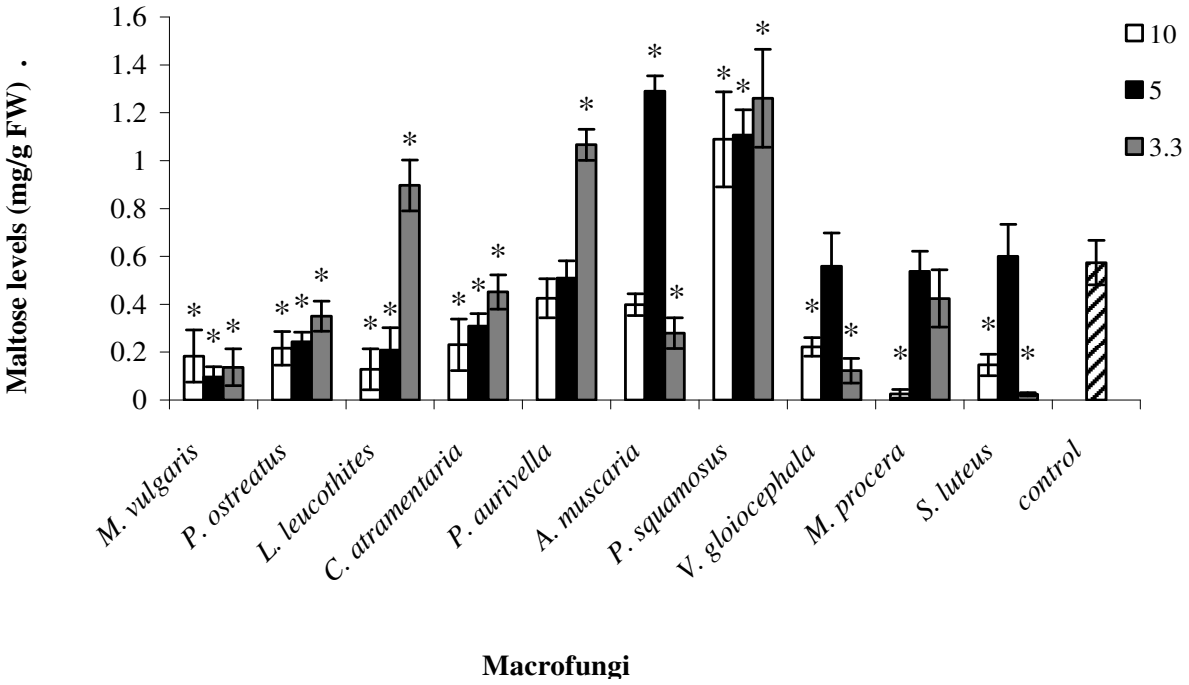

Figure 7. The effect of macrofungi extracts on maltose levels in maize plant.

2004). Fungi are well recognized for their ability to produce diverse biologically active metabolites (Saxena and Pandey, 2001). Some fungal metabolites are known to have allelopathic effects on cell division, germination and specific enzyme function (Magan, 2001). The provided data showed that the effects of MFE on germination depend on the MF species and concentration of the extract.

Plant grains metabolize starch, proteins and fatty acids to provide energy and synthesize the enzyme as well as

Table 1. The effect of macrofungi extracts on linoleic and oleic acid levels in maize plant.

Macrofungi	Linoleic acid levels (µg/g FW)			Oleic acid levels (µg/g FW)		
	10%	5%	3.3%	10%	5%	3.3%
M. vulgaris	116.00*	12.70	8.46*	-	-	-
P. ostreatus	4.00	710.30*	540.60*	21.30*	99.60*	141.00*
L. leucothites	11.13	52.30*	16.30	115.30*	25.30*	1.19
C. atramentaria	533.00*	107.00*	466.30*	866.30*	141.60*	337.30*
P. aurivella	35.00	19.30*	7.30	21.39*	-	-
A. muscaria	13.30*	78.30*	96.00*	-	-	-
P. squamosus	300.30*	112.30*	232.00*	0.20	130.30*	0.30
V. gloiocephala	840.00*	2.50	13.00	379.60*	-	-
M. procera	12.76*	1.10	0.21	-	-	-
S. luteus	4.97	330.60*	74.60*	-	-	-
Control		2.00			0.57	

the necessary compounds for growth and development. The higher level of the compounds might be due to the result of metabolic pathway suppression which does not allow metabolizing synthesized compounds; or the allelochemicals in macrofungi extract may stimulate the synthesis of the compounds of which levels were found to be higher in tested grains than that of the control. Besides, the higher carbohydrate level may be attributed to cell wall metabolizing enzymes which degrade oligosaccharide to release the small sugars (Grant Reid, 1997). The most stimulative effect of MF extract was observed in LA synthesis whereas the OA levels were tremendously inhibited by MFE. It was reported that the changing level of fatty acids affects the turnover of membrane lipid composition, the fluidity, elasticity and mobility of bilayer as plant answer to stress (Svenningsson et al., 1990; Surjus and Durand, 1996). Unfortunately we do not have the chance to compare our results with the previous studies, because of the limited reports on the allelopathic effects of macrofungi on plants.

In general, it might be stated that the macrofungi extract inhibited the germination and physiological processes of maize grain. However, some stimulating effects on these parameters were also determined. It could be concluded that although some of the macrofungi allelochemicals have stimulating effects, others are inhibitive. The allelochemicals are needed to be characterized and tested on plants in order to determine inhibitive and stimulative substances which might be used as plant growth regulators, herbicide or insecticide an alternative to synthetic compounds.

When germination, plumule and radicle length and sugar and fatty acids concentrations are considered together, the effects of MFE showed similar pattern on the parameters. The MFE may be tested on economically important plant seeds using this model system and has great potential to be used for controlling dormancy, senescence, cell division and physiological processes in organic and sustainable agricultural systems where synthetic herbicides are not allowed.

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