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Response of *Achlya racemosa, A. proliferoides* and *Saprolegnia furcata* to Sub-lethal Treatments of Amino Acids

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The effect of four sub-lethal concentrations (400, 800, 1,200 and 1,600 μg/ml) of three amino acids such as isoluecine, aspartic acid and phenylalanine on vegetative growth and sexual and asexual reproduction of *Achlya racemosa, A. proliferoides* and *Saprolegnia furcata* was investigated. The density of vegetative growth and diameters of vegetative colonies of species of the Oomycetes fungi decreased with rising the concentration of the applied amino acid. Vegetative hyphae of treated fungi almost appeared branched in case of *S. furcata*, thick in case of *A. racemosa* and distorted in case of *A. proliferoides* as compared with control. The different treatments with amino acids depressed both sporangial formation and discharge, which were dependent on the tested species of zoosporic fungi, the amino acid and its dosage. Phenylalanine was the most effective amino acid in inhibiting sporulation and *S. furcata* was the most sensitive fungal species. Aspartic acid and isoleucine stimulated germination of discharged spores through the formation of germlings. Gemmae formation by the three fungi was reduced at the low concentrations of amino acids and nearly missed at high concentrations. Sex organs (oogonia and antheridia) were affected partly; rudiment oogonia were observed at low concentrations (400 and 800 μg/ml) and disappeared at higher concentrations, whereas antheridial branch formation was stimulated as the fungi were treated with isoleucine and to some extent phenylalanine.

KEYWORDS: Amino acids, Saprolegniaceae, Sex organs, Sporulation, Zoosporic fungi

Most species of zoosporic fungi live as saprophytes on seeds, grains and decaying plant and animal materials (Sparrow, 1960; Dick, 1968). However, some zoosporic fungal genera and species are pathogenic for fish species inhabiting freshwater habitats. These fungi can attack fish species through turnover and hydrolysis their proteinaceous organic matter to simple amino acids, which were released in the ecosystem. Numerous works (Papavizas and Davey, 1960; Gleason, 1970, 1973; Herr, 1973; Nolan, 1975, 1983) enable to utilize amino acids as carbon and nitrogen sources by some species of zoosporic fungi in synthetic media. However, nearly no attention has been given for studying the effects of amino acids on vegetative growth, and sexual (oogonia and antheridia) and asexual (zoosporangial formation and discharge and gemmae formation) reproduction of zoosporic fungi belonging to Saprolegniaceae. So, this investigation was adopted for studying the effects of various levels of three representative amino acids; isoleucine (aliphatic), aspartic acid (dicarboxylic) and phenylalanine (aromatic) on vegetative growth, sporulation, gemmae formation and sexual reproduction of three zoosporic fungal species namely *Achlya racemosa, A. proliferoides* and *Saprolegnia furcata* which were of common occurrence in water of the River Nile system in Egypt (El-Hissy, 1979; El-Hissy *et al.*, 1982; El-Hissy and Khallil, 1989) and were also associated with some fish species worldwide (Scott and O'Bier, 1962; Olah and Farkas, 1977; Srivastava and Srivastava, 1977; El-Hissy *et al.*, 1989; Hatai *et al.*, 1990; Durborow *et al.*, 1991; Noga, 1993).

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Materials and Methods

Tested zoosporic fungi. Three zoosporic fungal species namely, *A. racemosa*, *A. proliferoides* and *S. furcata* which belong to family Saprolegniaceae, Oomycetes were tested during this investigation. The tested species were pathogenic agents capable of infecting some fish species allover the world (Scott and O'Bier, 1962; Olah and Farkas, 1977; Srivastava and Srivastava, 1977; El-Hissy *et al.*, 1989; Hatai *et al.*, 1990; Durborow *et al.*, 1991; Noga, 1993).

Medium (Water-sesame seeds culture). Sterilized germinating sesame seeds were introduced into sterilized Petri-dishes (10 cm diameter each) containing sterilized, filtered and distilled Nile water (20 m*l* each).

Amino acids. Three amino acids belonging to different groups were applied and these were isoleucine (aliphatic), aspartic acid (dicarboxylic) and phenylalanine (aromatic). These amino acids were prepared in four different concentrations (400, 800, 1,200 and 1,600 μg/m*l*). These concentrations were sub-lethal for the tested species of zoosporic fungi where these fungi cannot tolerate concentrations higher than 1,600 μg/m*l* of the three amino acids as determined through preliminary experiments. Also, these con-

centrations were found to be the most effective to perform the purpose of this study.

Inoculation, incubation and examination. Fungal inoculation was made by means of suspension of zoospores. Zoospore suspension was adopted by growing the tested three fungi on sesame seeds water culture in sterilized Petri-dishes, each containing 20 m*l* distilled and filtered Nile water. The prepared Petri-dishes were incubated at 20±2°C for eight days for zoosporangial discharge and zoospore production. Three ml of zoospore suspension of each species was pipetted under aseptic conditions and was used as inoculum for sterilized Petri-dishes which contain sesame seeds-water culture previously adjusted to give different concentrations (0, 400, 800, 1,200 and 1,600 μg/m*l*) of each of the three amino acids. Three replicates of Petri plates were used for each amino acid concentration and tested fungal species. Thereafter, Petri-dishes were incubated at 20±2°C. Observations and microscopical examinations on the growing colonies were started from the second day and subsequently extended for three weeks during which any deviation from normal shapes (at control) were observed, described and recorded. The morphological remarks of each tested species of zoosporic fungi included developing vegetative hyphae, zoosporangial formation and discharge, sexual reproductive organs (oogonia and antheridia) and gemmae formation (asexual structures). The approximate number of sporangia, discharged sporangia, oogonia and antheridia and gemmae per one seed was assessed by counting after removal of the culture medium with sterilized pipette and expressed as high, moderate, low and rare rates as follows:

A - In case of *A. racemosa*: high rate (more than 20/seed), moderate rate (10~20/seed), low rate (5~9/seed) and rare rate (less than 5/seed).

B - In case of *A. proliferoides*: high rate (more than 12/seed), moderate rate (6~12/seed), low rate (3~5/seed) and rare rate (less than 3/seed).

C - In case of *S. furcata*: high rate (more than 15/seed), moderate rate (7~15/seed), low rate (3~6/seed) and rare rate (less than 3/seed).

Most of the microscopical observations were photographed using Olympus microscope provided with Olympus camera and were compared with that at control. Diameters of radiating hyphae of each tested species of zoosporic fungi around sesame seeds were also measured.

Results

Effect of phenylalanine. Generally, the density of vegetative growth and the diameters of vegetative colonies of the tested three species of zoosporic fungi were decreased as the sub-lethal concentration of phenylalanine increased (Table 1). Only sterile vegetative hyphae appeared in case of *S. furcata* treated with 1,200 and 1,600 μg/m*l* of phenylalanine. The vegetative hyphae of *A. racemosa* were observed delicate at 1,600 μg/m*l* of phenylalanine. In case of *A. proliferoides*, the hyphae were distorted at 1,600 μg/m*l* of phenylalanine and in general the density of vegetative hyphae was more vigorous at different treatments of phenylalanine as compared with isoleucine.

Regarding *A. racemosa*, low rate of discharged sporangia appeared at 400 μg/m*l* of phenlalanine, whereas sporangia and sporangial discharge at 800 μg/m*l* were rarely formed (Table 2). Only rare rate of non-discharged sporangia was observed at 1,200 μg/m*l* of phenylalanine and sporangia were totally missed at 1,600 μg/m*l*. With respect to *A. proliferoides*, sporangia and sporangial discharge appeared in low rates at 400 μg/m*l* of phenylalanine whereas sporangia rarely formed at 800 μg/m*l* and these sporangia showed no discharge at all. Only non-discharged, deformed sporangia were observed rarely at 1,200 μg/m*l* of phenylalanine and no sporangia appeared at 1,600 μg/m*l*. In case of *S. furcata*, low rate of differentiated sporangia was remarked at 400 μg/m*l* of phenylalanine and these sporangia showed no spores liberation. Sporangia (non-discharged) rarely observed at 800 μg/m*l* and neither sporangia nor discharge were shown at 1,200 and 1,600 μg/m*l*.

With regard to *A. racemosa*, the gemmae highly occurred at 400 μg/m*l* of phenylalanine and were rare at 800

Table 1. Effect of various sub-lethal concentrations of the applied amino acids such as phenylalanine, aspartic acid and isoleucine on diameters (cm) of vegetative colonies of *Achlya racemosa*, *A. proliferoides* and *Saprolegnia furcata* as measured after ten days of incubation using water-sesame seeds cultures at 20±2°C

Concentration μg/m*l*	Fungal species								
	Achlya racemosa			*Achlya proliferoides*			*Saprolegnia furcata*		
	PA[a]	AA	IL	PA	AA	IL	PA	AA	IL
0.0	1.8	1.8	1.8	2.5	2.5	2.5	2.1	2.1	2.1
400	1.6	1.4	1.6	2.1	2.4	1.7	1.8	2.1	2.0
800	1.2	1.3	1.6	1.9	1.8	1.5	1.3	0.8	2.1
1200	1.1	1.0	1.3	1.2	1.6	1.1	0.8	0.6	1.5
1600	0.6	0.8	0.9	0.9	1.2	0.7	0.8	0.5	0.8

[a]PA = Phenylalanine, AA = Aspartic acid, IL = Isoleucine.

Table 2. Effect of various sub-lethal concentrations of the applied amino acids such as phenylalanine (PA), aspartic acid (AA) and isoleucine (IL) on sporangial formation, zoosporangial discharge, sex organs and gemmae formation by *Achlya racemosa*, *A. proliferoides* and *Saprolegnia furcata* using water sesame seeds culture at 20±2°C

Aspect	Concent-ration (μg/ml)	Fungal species[a]								
		Achlya racemosa			*A. proliferoides*			*Saprolegnia furcata*		
		PA	AA	IL	PA	AA	IL	PA	AA	IL
Sporangial formation	0.0	H	H	H	H	H	H	H	H	H
	400	L	L	M	L	M	M	L	M	M
	800	R	R	M	R	L	L	R	R	M
	1200	R	–	L	R	R	L	–	–	H
	1600	–	–	R	–	R	L	–	–	R
Sporangial discharge	0.0	H	H	H	H	H	H	H	H	H
	400	L	–	L	L	M	M	–	M	M
	800	R	–	L	–	L	R	–	–	L
	1200	–	–	R	–	R	R	–	–	H
	1600	–	–	–	–	–	R	–	–	R
Sex organs formation	0.0	H	H	H	H	H	H	H	H	H
	400	H	L	H_aR_o	R_a	R	R_a	–	M_o	M_o
	800	R_o	R_a	H_a	R_a	–	–	–	R_o	R_o
	1200	R_a	–	H_a	R_a	–	–	–	–	–
	1600	–	–	R_o	–	–	–	–	–	–
Gemmae formation	0.0	H	H	H	H	H	H	H	H	H
	400	H	–	–	H	R	H	–	H	–
	800	R	–	–	M	R	R	–	H	–
	1200	–	–	–	R	R	–	–	H	–
	1600	–	–	–	R	–	R	–	–	–

[a]H = High rate, M = Moderate rate L = Low rate and R = Rare rate as follows:
In case of *A. racemosa*: H; >20, M; 10~20, L; 5~9 and R; <5 cases/seed.
In case of *A. proliferoides*: H; >12, M; 6~12, L; 3~5 and R; <3 cases/seed.
In case of *S. furcata*: H; >15, M; 7~15, L; 3~6 and R; <3 cases/seed.
– = Absent, M_o = Moderate oogonia, R_o = Rare oogonia, H_a = high density of antheridia, R_a = Rare antheridia.

μg/ml as shown in Table 2. No gemmae appeared at all at 1,200 and 1,600 μg/ml of phenylalanine. High rate of *A. proliferoides* elongated gemmae was formed at 400 μg/ml of phenylalanine whereas gemmae were moderately occurred at 800 μg/ml and they assumed variable shapes (semicircular and elongated). At 1,200 and 1,600 μg/ml of phenylalanine, gemmae were observed in rare rate. *S. furcata*, showed no gemmae formation at all at any applied concentration of phenylalanine.

As presented in Table 2, *A. racemosa* supplemented with phenylalanine showed high rate of mature oogonia and antheridia at 400 μg/ml whereas only rare rate of papillate and lemon-shaped abortive oogonia (Fig. 7) appeared at 800 μg/ml. Only non-functional swollen antheridia were remarked rarely at 1,200 μg/ml of phenylalanine and sexual reproductive organs were absent at 1,600 μg/ml. With regard to *A. proliferoides*, only non-functional antheridia were observed rarely at 400, 800 and 1,200 μg/ml of phenylalanine and sex organs were missed at 1,600 μg/ml. In case of *S. furcata*, no sex organs (neither oogonia nor antheridia) appeared at any concentration of phenylalanine.

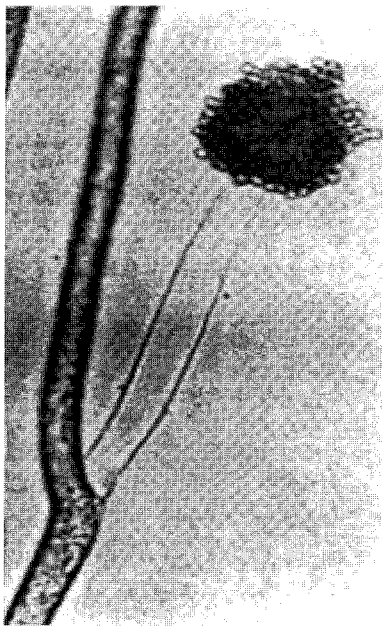

Fig. 1. Discharged and encysted zoospores of *A. proliferoides* at control.

Effect of aspartic acid. The density of growing vegetative hyphae and the diameters of vegetative colonies of the three zoosporic fungal species were decreased with rising

the dose concentration of aspartic acid (Table 1) throughout the period of incubation. The vegetative hyphae of *A. racemosa* were obviously branched at 800 μg/ml of aspartic acid as compared with that at control and the hyphae were tumoured at 1,200 and 1,600 μg/ml. Regarding *A. proliferoides*, a distinct protoplasmic accumulation (Fig. 8) was observed throughout the vegetative hyphae at 800 and 1,200 μg/ml of aspartic acid. At 1,600 μg/ml, the vegetative hyphae were distorted and showed severe protoplasmic accumulation. No great morphological changes in *S. furcata* vegetative hyphae treated with aspartic acid regardless its concentration.

With regard to *A. racemosa*, low rate of differentiated and non-discharged sporangia was observed at 400 μg/ml of aspartic acid comparable to untreated control (Table 2). Only abortive and non-discharged sporangia were counted rarely at 800 μg/ml of aspartic acid and sporangia were totally absent at 1,200 and 1,600 μg/ml. *A. proliferoides* sporangia and sporangial discharge occurred in moderate rate at 400 μg/ml of aspartic acid as compared with control and non-discharged sporangia showed mature spores which geminated within their sporangia. Low rate of discharged sporangia was observed at 800 μg/ml and discharged spores germinated directly after liberation and encystment (Fig. 2). Sporangia appeared rarely at 1,200 μg/ml and these sporangia differentiated into mature spores, which either geminated within sporangia or in front of it after liberation. Only rare rate of undifferentiated and non-discharged sporangia was remarked at 1,600 μg/ml. Sporangia and sporangial discharge in case of *S. furcata* appeared moderately at 400 μg/ml of aspartic acid as compared with control whilst only rare rate of differentiated and non-discharged sporangia was observed at 800 μg/ml. Sporangia were missed at 1,200 and 1,600 μg/ml.

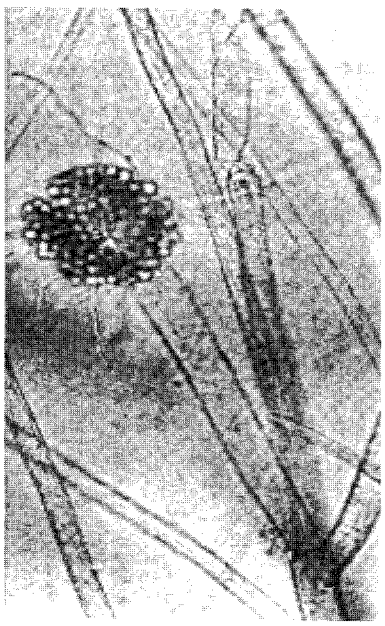

Fig. 2. *Achlya proliferoides* sporangium showing germlings formation at the apical pore as appeared at 800 μg/ml of aspartic acid.

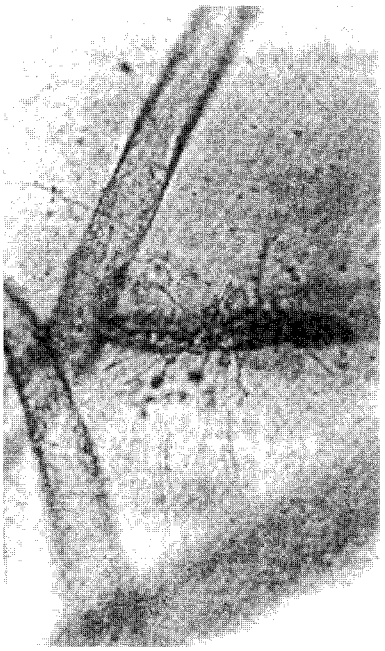

Fig. 3. *Achlya racemosa* with germinated spores within sporangium at 1,200 μg/ml of isoleucine.

In case of *A. racemosa*, no gemmae were observed at different applications of aspartic acid (Table 2). The gemmae of *A. proliferoides* reacted with the different treatments of aspartic acid. Gemmae occurred rarely and appeared connected with wire-like structure at 400 μg/ml as compared with that at control whereas gemmae were observed in zigzag fashion (Fig. 11) and rare rate at 800 μg/ml. Gemmae (deformed) rarely formed at 1,200 μg/ml and were missed at 1,600 μg/ml. *S. furcata* gemmae were observed and counted in high rates at 400, 800 and 1,200 μg/ml of aspartic acid and were totally absent at 1,600 μg/ml.

With regards to *A. racemosa*, low rate of differentiated, sessile oogonia and twined antheridia appeared at 400 μg/

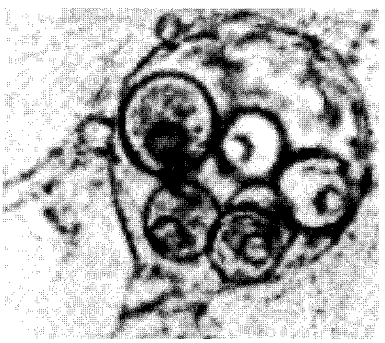

Fig. 4. *Achlya proliferoides* sex organs at control showing eccentric oospores.

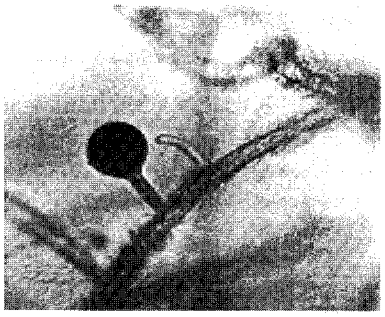

Fig. 5. Rudiment oogonia and non-functional antheridia of *Achlya proliferoides* at 400 μg/ml of aspartic acid.

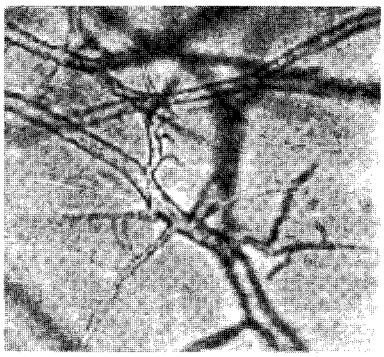

Fig. 6. Long antheridial branches in case of *Achlya racemosa* treated with 1,200 μg/ml of isoleucine.

ml of aspartic acid as compared with the control. At 800 μg/ml, only rare rate of non-functional antheridia was observed whilst sex organs were completely disappeared at 1,200 and 1,600 μg/ml. In case of *A. proliferoides*, only non-functional antheridia and immature oogonia (Fig. 5) appeared in rare rate at 400 μg/ml of aspartic acid relative to untreated control whereas sexual reproductive structures were absent at the higher three tested concentrations. Moderate rate of *S. furcata* differentiated oogonia was observed at 400 μg/ml of aspartic acid as compared with the control while oogonia were undifferentiated and rarely formed at 800 μg/ml. No sex organs formed at all at 1,200 and 1,600 μg/ml of aspartic acid.

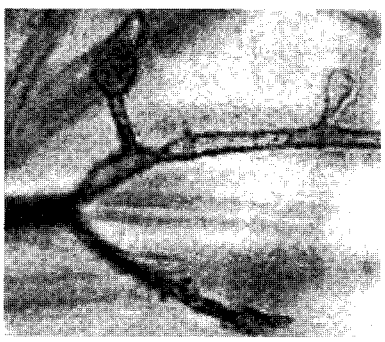

Fig. 7. Lemon-shaped, abortive oogonia of *Achlya racemosa* at 800 μg/ml of phenylalanine.

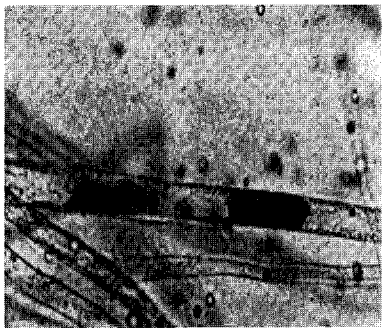

Fig. 8. *Achlya proliferoides* vegetative hyphae treated with 1,200 μg/ml of aspartic acid showing protoplasmic accumulation at various distances.

Effect of isoleucine. The density of vegetative growth and the diameters of vegetative cultures of the three species of zoosporic fungi were decreased with rising the treatments of isoleucine as presented in Table 1. The thickness of *A. racemosa* vegetative hyphae was decreased with the rise of isoleucine concentration. In case of *A. proliferoides*, the hyphae were distorted at 1,600 μg/ml of isoleucine and in general the density of vegetative hyphae was less vigorous at different treatments of isoleucine as compared with phenylalanine. The developing vegetative hyphae of *S. furcata* were thin and appeared laterally branched at 400 and 800 μg/ml of isoleucine as compaed with control. Vegetative growth was restricted at 1,200 and 1,600 μg/ml.

The data presented in Table 2 indicate that the sporangia of *A. racemosa* moderately occurred at 400 and 800 μg/ml of isoleucine and showed low rates of discharge as compared with the control. Low rate of cleavage sporangia and rare sporangial discharge appeared at 1,200 μg/ml

Fig. 9. *Achlya proliferoides* gemmae at control.

and both discharged and non-discharged spores germinated forming germlings (Fig. 3). Only immature, non-discharged sporangia were remarked rarely at 1,600 μg/ml. *A. proliferoides* sporangia and sporangial discharge appeared in moderate rate at 400 μg/ml of isoleucine and discharged spores showed germination. At 800 μg/ml, low rate of differentiated sporangia and rare discharge appeared and differentiated spores germinated via germ tubes. Sporangia were also observed in low rate and discharged rarely without showing spore germination at 1,200 and 1,600 μg/ml. Moderate rate of *S. furcata* sporangia and sporangial discharge and proliferation appeared at 400 μg/ml of isoleucine. Sporangia also moderately counted at 800 μg/ml but they showed low discharge and proliferation. Sporangia as well as sporangial discharge and proliferation were remarked in high rate at 1,200 μg/ml although the restriction of vegetative growth whereas they observed rarely at 1,600 μg/ml.

In case of *A. racemosa* and *S. furcata*, gemmae formation was very sensitive for the different supplements of isoleucine since no gemmae appeared at any concentration. *A. proliferoides*, gemmae were highly occurred and of elongated shape at 400 μg/ml of isoleucine and this dosage stimulated the differentiation of gemmae into zoospores, which geminated inside their gemmae (Fig. 10). Gemmae (deformed) rarely formed at 800 and 1,600 μg/ml of isoleucine and disappeared at 1,200 μg/ml.

Regarding *A. racemosa*, high density of twined antheridia and rare rate of abortive oogonia was observed at 400 μg/ml of isoleucine relative to untreated control (Table 2). Only high density of twined antheridia (Fig. 6) appeared at 800 and 1,200 μg/ml of isoleucine and these antheridia rarely formed at 1,600 μg/ml. In case of *A. proliferoides*, only rare rate of swollen antheridia was observed at 400 μg/ml of isoleucine and sex organs were missed at the other tested concentrations. Only moderate rate of *S. furcata* differentiated oogonia was remarked at 400 μg/ml of isoleucine as compared with the control while only undifferentiated oogonia appeared rarely at 800 μg/ml. Neither oogonia nor antheridia were formed at the higher concentrations (1,200 and 1,600 μg/ml) of isoleucine.

Fig. 10. *Achlya proliferoides* gemmae showing zoospores differentiation and germination at 400 μg/ml of isoleucine.

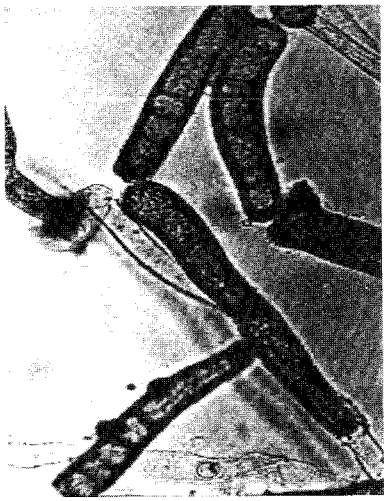

Fig. 11. Zigzag-shaped gemmae of *Achlya proliferoides* as appeared at 800 μg/ml of aspartic acid.

Discussion

The results obtained from this study indicated that the colony diameter of vegetative cultures of species of zoosporic fungi was decreased with rising the dosage of each of the three applied amino acids. Allen and Hussey (1970) also found that L-cysteine inhibited the linear growth of *Helminthosporium carbonum* by 20% at pH value 5.5 and 60% at pH 4. In addition, Prasad and Chaudhary (1977) mentioned that DL-alanine and DL-valine added as an extra nitrogen for fortifying the already present inorganic nitrogen source actually acted as growth retardant for *Fusarium oxysporum* f. sp. *udum*. Moreover, De Lucca *et al.* (1996) reported that N-acetylcysteine inhibited hyphal growth of *Aspergillus* spp. and *Fusarium* spp.

The growing vegetative hyphae of *A. racemosa* and *S. furcata* were branched by low concentrations (400 and 800 μg/ml) of aspartic acid and isoleucine, respectively. Youatt (1985) also found that the multiple hyphal branching occurred when *Allomyces macrogynous* transferred to glucose-histidine-methionine solution. Harold and Harold (1986) reported that hyphae of *Achlya bisexualis* growing on a medium deficient in amino acids were elongated but produced relatively low branches. Schreurs *et al.* (1989), reported that arginine is a good inducer of *Achlya bisexualis* branching whereas methionine was only a fair branching agent.

The vegetative hyphae of *A. racemosa* treated with either isoleucine or aspartic acid were thicker as compared with control. Fonvieille *et al.* (1989) found that cell thickness of *Scopulariopsis brevicaulis* hyphae grown on synthetic amino acid supplemented medium increased as compared with those grown on a control medium. On the other hand, *A. proliferoides* vegetative hyphae treated with aspartic acid showed severe protoplasmic accumulation especially at higher concentrations. Accordingly, Faro (1971) observed that vegetative hyphae of *Achlya heterosexualis* treated with proline were not laden with cytoplasm.

Generally, amino acid applications inhibited both sporangial formation and discharge with variable degrees. Inhibition of sporulation was dependent on the tested fungal species, the applied amino acid, and its concentration. The effect of aspartic acid on inhibiting sporulation and discharge was more pronounced in case of *A. racemosa* and *S. furcata* as compared with *A. proliferoides*. Phenylalanine was the most effective amino acid in inhibiting sporulation and *S. furcata* was the most sensitive fungal species of the three fungi tested. Also, it was found that isoleucine was the less effective of the three tested amino acids on sporulation and sporangial discharge of the three species of zoosporic fungi; sporangia and sporangial discharge were observed at all tested concentrations including the highest levels. Inhibition of sporulation in the three fungi increased with rising the concentration of the three applied amino acids.

Prasad and Chaudhary (1977) noted that the sporulation of microconidia in *Fusarium oxysporum* f. sp. *udum* was indifferently affected by DL-alanine and DL-valine. DL-valine stimulated microconidial formation in young cultures only. In both young and old cultures, the lowest concentration of DL-valine depressed macroconidial sporulation. On the other hand, DL-alanine depressed both macro- and microconidial sporulation. DL-isoleucine, on the other hand, stimulated sporulation of macro- and microconidia. Ducan and Himelick (1986) reported that conidial production of *Verticillium dahliae* varied greatly depending on the amino acid used as the nitrogen sources in Czapek's-Dox medium. Moreover, Oritsejafor (1986) found that *Fusarium oxysporum* f. sp. *elaeidis* showed good sporulation on DL-leucine while sporulation was poor on L-asparagine and DL-aspartic acid. However, Gunasekaran and Weber (1972) reported that the addition of tryptophan or methionine stimulated sporulation in *Rhizopus arrhizus*.

The response of encysted spores for germination and forming germlings either in front of empty sporangia or within their sporangia was more pronounced in case of *A. proliferoides* than *A. racemosa* and appeared to be dependent on applied amino acid and tested organism. Both aspartic acid (more strongly) and isoleucine were the most effective to induce germination of liberated spores, but no effect was found in case of phenylalanine. This observation was usually increased with rising the concentration of aspartic acid and isoleucine up to certain limit in spite of depression in the number of counted sporangia. However, this phenomenon did not observe absolutely in case of *S. furcata* at any applied concentration of the three amino acids.

Rai and Strobel (1966) reported the amino acid fraction of root exudates stimulated the germination of *Aphanomyces cochlioides* zoospores. Abdel-Rahim and Arbab (1985) found that glutamic acid and valine were the most effective nitrogenous compounds for spore germination of *Aspergillus niger*. However, De Lucca *et al.* (1996) indicated that N-acetylcysteine inhibited germination of conidia of *Aspergillus* spp. and *Fusarium* spp. Also, Zhou *et al.* (1996) found that DL-histidine did not affect significantly the germination of *Epicoccum nigrum* conidia, but L-phenylalanine reduced germination. Daigle and Cotty (1991) indicated that cysteine inhibited the germination of *Alternaria* spp. conidia. They concluded that cysteine may reduce conidial wall permeability to nutrients, thereby affecting germination. In addition, Johri and Panday (1980) found that cysteine inhibited germination of sporangiospores of *Rhizopus rhizopodiformis*.

Gemmae formation by the three species of zoosporic fungi were observed in variable rates (high rare) at the low treatments (400 and 800v) of the applied amino acids whereas they were almost absent at higher concentrations. Gemmae formation in response to different treatments of amino acids was more sensitive in case of *S. furcata* as compared with *A. racemosa* and *A. proliferoides*. *A. proliferoides* gemmae exhibited morphological alterations at the low concentrations of the three amino acids and the lowest concentration (400 μg/ml) of isoleucine induced its gemmae differentiation into zoospores with subsequent germination.

More or less similar observations were obtained by Kritzman and his colleagues (1976) who found that L-threonine (10^{-2} M) favored formation of sclerotia of the fungus *Sclerotium rolfsii* but glycine (10^{-1} M) inhibited sclerotium formation. Mantle and Nisbet (1976) found that aspartic acid and glutamic acid supported differentiation of plectenchymatic sclerotia of *Claviceps purpurea* whereas lysine supplied exogenously as a nitrogen source did not promote sclerotial differentiation. In addition, Prasad and Chaudhary (1977) observed that the lowest concentration of valine stimulated chlamydospore differentiation of *Fusarium oxysporum* f. sp. *udum* in old cultures, higher concentrations being less effective while DL-alanine did not invigorate chlamydospore formation.

Sexual reproductive organs responded variably as a result of amino acids treatments and that is depended mainly on the tested species of zoosporic fungi and the type of the applied amino acid and its concentration. In

case of *A. proliferoides*, sex organs nearly represented by non-functional antheridia, which were almost appeared at the low concentrations of amino acids. Aspartic acid was the most effective amino acid in suppressing sex organ formation of *A. racemosa* whereas isoleucine stimulated the appearance of twined and very long antheridial branches and this phenomenon was observed somewhat in less extent in case of phenylalanine. Oogonia were sensitive for the various treatments of amino acids and were almost appeared at the low concentrations where they were rudiment and abortive. *Saprolegnia furcata* treated with phenylalanine failed to produce sexual reproductive organs at any amended concentration. Both isoleucine and aspartic acid had a similar effect on sex organs formation of *S. furcata* which were represented only by oogonia. Oogonia were differentiated into oospores and were of moderate number at 400 μg/ml, undifferentiated and rare at 800 μg/ml and were absent at 1,200 and 1,600 μg/ml.

Similarly, Leal and Gomez-Miranda (1967) reported that the suitability of glucose-amino acid media for the production of oospores by *Phytophthora heveae* was decreased as the concentration of the amino acids was increased. Also, Faro (1971) found that proline inhibited the differentiation of *Achlya heterosexualis* oogonia and their development ceased shortly after the formation of oogonial initials. The oogonial initials failed to become densely laden with cytoplasm and therefore, they lacked the dark coloration characteristic of normally developing oogonia. Chiu and Moore (1988) noted that glutamine was able to inhibit basidium differentiation in *Coprinus cinereus*. However, Eckert *et al.* (1999) observed that four tryptophan auxotrophic strains of *Aspergillus nidulans* are sterile on medium containing low tryptophan concentrations. Fruit-body formation was restored by supplementation with high concentration of tryptophan.

It can be concluded from this study that various effects of sub-lethal doses of the applied amino acids such as phenylalanine, aspartic acid and isoleucine on the Oomycetes fungi inhibited vegetative growth, asexual reproduction (sporulation and gemmae formation) and sex organ formation. Of the three amino acids, aspartic acid and isoleucine stimulated germination of either discharged or non-discharged spores and induced clear germling formation in case of *A. racemosa* and *A. proliferoides*. Also, these amino acids stimulated the protoplasmic differentiation of gemmae into zoospores which subsequently germinating with the formation of germ tube. These results may give interpretation for the isolation of species of aquatic fungi pathogenic for fish species as non-sexual isolates in most cases. This may be due to the ability of these species of zoosporic fungi to hydrolyze fish protein matter into simple amino acids via protease enzyme secretion, then they morphologically affected by these products.

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