In recent years, artificial diets have been developed for many lepidopterous caterpillars of agricultural pests[1–6]. However, efforts made at this Institute in this direction with the wheat germ medium (personal communication) met with only partial success, as earlier instars of the insect had to be reared on sugarcane tissues. Rearing of only the last 2 instars of the pest was possible with artificial medium. Therefore, a number of new recipes were tried and of these one with French beans (*Phaseolus vulgaris* L.) was found to be suitable for the artificial rearing of *Chilo auricilius*, right from the 1st instar caterpillars to developing insects.

Material and method. French bean medium comprises of: French bean, 100 g; yeast extract, 16 g; ascorbic acid, 1 g; casein, 50 g; Wesson's salt, 5 g; sorbic acid, 0.5 g; methyl-*para*-hydroxybenzoate, 1 g; vitamin mixture[7], 8 g; agar-agar, 8 g; powder of dried sugarcane tops, 50 g; formaldehyde, 1.0 ml, and water, 425 ml.

French beans, first soaked in tap water for 24 h, were boiled for a few min and seed coats were peeled off. Peeled seeds were mixed with other ingredients, except agar-agar, ascorbic acid, sugarcane powder and vitamins, and ground to a paste in hand pastle and mortar. Agar-agar was melted in 155 ml of water and sugarcane powder was slowly added to it accompanied by constant stirring. Bean paste, suspended in 240 ml of water was then mixed slowly and thoroughly into molten agar medium, stirred thoroughly and cooked for 5 min on a water-bath. Vitamin mixture and ascorbic acid were dissolved separately in 30 ml of distilled water. Along with formaldehyde, this was finally added to the diet on cooling.

Rearing technique. Sterilized 9.5 cm × 2.5 cm glass tubes, open end plugged with sterilized cotton, were used for the rearing of the caterpillars. The diet was poured into the tubes with the help of a glass rod, upto a quarter of the space, and allowed to set in a slanting position. The tubes were placed in a wire basket and kept overnight for the removal of moisture from the inner walls.

Freshly hatched stalk borer larvae were transferred to the medium with a surface sterilized fine camel-hair brush, at the rate of 10 per tube. After 2–3 weeks these were transferred to fresh tubes keeping 5 larvae per tube. After 33–58 days, these pupated either near the cotton plug or within the medium after forming a pupal cell. The pupae were taken out and kept in 7.5 cm diameter petri-plates, where after 6–10 days, adults emerged.

Range of duration in days, of various stages and fecundity of stalk borer moths reared on an artificial diet.

Larval period	Pupation (%)	Pupal period	Moth emergence (%)	Longevity ♂	Longevity ♀	Eggs laid per ♀	Sex ratio ♂ : ♀
33–38 (47.0)	82.0	6–10 (8.3)	65.8	5–6	4–5	134–265 (224.8)	1 : 0.8

Figures in parentheses are the averages.

Moths were mated in 15 cm × 10 cm diameter round glass jars and allowed to oviposit on freshly cut 15 cm long sugarcane leaf sections. The rearing was done in a constant temperature room maintained at 22 ± 27 °C and 60–75% RH.

Results. Data obtained for one complete life cycle are presented in the Table. Contamination of medium by micro-organisms was also observed in certain cases; the infected tubes were discarded. The emerging moths were almost normal as regards their vigour and fecundity.

Zusammenfassung. Es wird eine Methode zur Zucht des Zuckerrohrstengelbohrers *Chilo auricilius* auf einem semisynthetischen Medium beschrieben.

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Indian Institute of Sugarcane Research, Lucknow-2 (India), 7 November 1972.

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Influence of Some Nitrogen Sources on Amino Acid Pool of Two Hyphomycetes

Evidence for the presence of amino acids in soil was provided by the studies on organic matter decomposition (MCLAREN and PETERSON[1]), roots excretions (VERONA[2]), metabolic activities and in situ decomposition of soil microflora.

In this connection, a study was made on the influence of 4 common nitrogen fertilizers on amino acid content of fungal mycelium and spent cultural media of 2 hyphomycetes isolated from soil.

This topic has received only a limited coverage in the literature, apart from some qualitative and partial data (STOKES and GUNNESS[3], WEETE, WEBER and LE TOURNEAU[4], WHITAKER and MORTON[5]), mostly referring to blastomycetes (MOAT, AHMAD and ALEXANDER and BARNES[6], BROWN[7], BROWN and ROSE[8]) or pathogenic species for man (KASHIAP, BISWAS and GHOSH[9]) or plants (CASTELLANI and CALLIANO[10], SHERROD and DOMSCH[11]). Recently many efforts have been made to define quantitatively the free amino acid pool of some micromycetes, with relatively uncertain results because of extraction

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Table I. Amino acid composition of mycelia (g × 100 g of mycelium dry wt.)

	Aspergillus niger				*Penicillium frequentans*			
	$NaNO_3$	NH_4NO_3	NH_2CN	$(NH_2)_2CO$	$NaNO_3$	NH_4NO_3	NH_2CN	$(NH_2)_2CO$
Basic								
Arginine	0.92	0.81	1.35	0.86	0.57	1.05	0.28	0.75
Histidine	0.39	0.30	0.47	0.32	0.22	0.41	0.15	0.42
Lysine	1.35	1.13	1.55	0.96	0.76	1.44	1.28	1.58
	(2.66)	(2.24)	(3.37)	(2.14)	(1.55)	(2.90)	(1.71)	(2.75)
Acidic								
Aspartic acid	1.81	2.65	2.18	2.47	2.45	2.56	2.16	2.88
Glutamic acid	3.12	2.97	3.50	1.89	2.61	2.24	1.74	2.31
	(4.93)	(5.62)	(5.68)	(4.36)	(5.06)	(4.80)	(3.90)	(5.19)
Neutral								
Glycine	0.95	0.96	1.15	1.02	1.10	1.19	1.15	1.19
α-Alanine	2.14	1.79	2.64	1.25	1.59	1.71	1.84	2.07
Valine	1.02	1.12	1.11	1.13	0.90	0.95	1.20	1.06
Leucine	1.50	1.13	1.74	1.58	1.49	1.64	1.78	1.64
Isoleucine	0.71	0.45	0.78	0.81	0.73	0.81	0.80	0.89
Phenylalanine	0.61	0.57	0.79	0.80	0.71	0.90	0.95	0.81
Tyrosine	0.50	0.62	0.73	0.67	0.59	0.64	0.76	0.64
Serine	1.12	1.23	1.34	1.23	1.49	1.60	1.36	1.60
Threonine	1.08	1.19	1.29	1.17	1.52	1.48	1.56	1.53
Proline	0.89	0.89	1.17	0.87	0.92	1.14	0.83	1.09
Methionine	0.30	0.43	0.47	0.38	0.17	0.32	0.50	0.32
Cystine	0.18	0.24	0.34	0.76	0.09	0.30	0.51	0.41
α-Aminobutyric acid	2.75	2.81	3.19	3.45	3.32	3.44	4.34	3.40
γ-Aminobutyric acid	2.10	2.24	2.38	2.36	2.40	2.25	2.71	2.15
	(15.85)	(15.67)	(19.12)	(19.95)	(17.02)	(18.37)	(20.29)	(18.80)
Total amino acids	23.44	23.53	28.17	26.45	23.63	26.07	25.90	26.74
NH_3	0.56	0.61	1.75	0.88	0.74	0.70	1.90	0.91
Total	24.00	24.14	29.92	27.33	24.37	26.77	27.84	27.65

Table II. Percentages of basic, acidic and neutral amino acids of mycelia

	Aspergillus niger				*Penicillium frequentans*			
	$NaNO_3$	NH_4NO_3	NH_2CN	$(NH_2)_2CO$	$NaNO_3$	NH_4NO_3	NH_2CN	$(NH_2)_2CO$
Basic	11.36	9.52	11.96	8.90	6.56	11.12	6.60	10.28
Acidic	21.03	23.88	20.16	16.48	21.41	14.57	15.06	19.13
Neutral	67.62	66.60	67.88	74.62	72.31	74.31	78.34	70.69
Total	100	100	100	100	100	100	100	100

difficulties (THORNTON and MCEVOY[12]). However, these difficulties seem to be overcome by the method of HEATHCOTE, DAVIES and HAWORTH[13].

Materials and methods. The microorganisms used were *Aspergillus niger* van Tieghem and *Penicillium frequentans* Westling, isolated from soil and maintained on potato dextrose agar slants. Their cultivation was performed in Czapek liquid medium containing 0.2‰ of nitrogen as: a) $NaNO_3$, b) NH_4NO_3, c) NH_2CN, d) $(NH_2)_2CO$.

After 15 days of static cultivation at 26 °C, the mycelium was filtered off, washed twice with known portions of deionized water and lyophilised. The spent cultural media were concentrated under vacuum, then evaporated to dryness at 80 °C.

The preparation of samples for amino acid analysis was as follows: 50 mg of mycelium were mixed in an ampoule with 10 ml of 6 N HCl. The ampoule was evacuated, sealed and incubated at 105 °C for 24 h. The hydrolysate was then evaporated to dryness using a rotary evaporator. The residue was resuspended in buffer, pH 2.2 and filtered. A liquid portion was finally analyzed in an automatic amino acid analyser ('Aminolyzer', Optica, Milano) as described by SPACKMAN, STEIN and MOORE[14]. The free amino acid pool of culture media was also analyzed after extraction with 85% boiling ethanol, in the same automatic apparatus.

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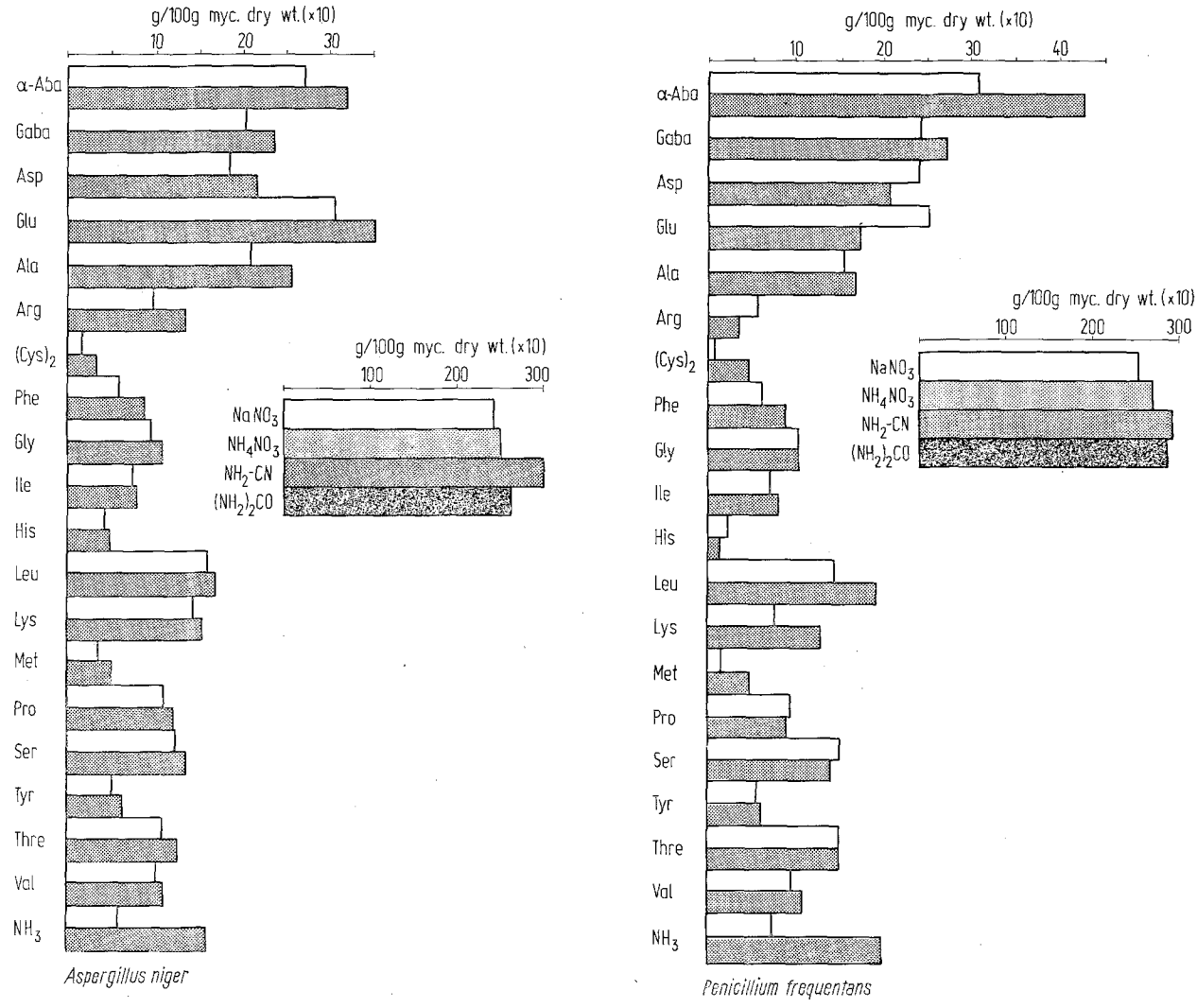

Aminoacidic content of the mycelium of *A. niger* and *P. frequentans*, in the presence of sodium nitrate and cyanamide.

Results and discussion. The amino acid composition of fungal mycelia of *A. niger* and *P. frequentans* cultivated with different nitrogen sources is given in Table I. Table II shows the relative percentage of basic, acidic and neutral amino acids.

As far as mycelia are concerned, there are no differences from a qualitative standpoint. On the other hand, one can observe some quantitative differences: *A. niger* shows larger amounts of basic and acidic amino acids than *P. frequentans*, as well as larger quantities of alanine, arginine and lysine (4.49 and 3.73 on an average, respectively).

More evident is the influence of the nitrogen source. The results indicate that ammonium salts (or compounds able to produce NH_3, as urea or cyanamide) give a higher level of amino acid content. This is particularly true for cyanamide. In its presence, for both microfungi, the amino acid content is higher than with $NaNO_3$ as nitrogen source. Of particular interest are the larger amounts of α- and γ-aminobutyric acids in *A. niger*, as well as of cystine and methionine: 0.48% with $NaNO_3$ and 0.81% with cyanamide in *A. niger*; 0.26% and 1.01%, respectively, in *P. frequentans*.

As far as the free amino acid pool of culture media is concerned, both micromycetes have shown an analogous behaviour, i.e. independence from the nitrogen source; in every case, citrulline and α-alanine have been found both for *A. niger* and *P. frequentans*.

Riassunto. E' stata determinata la composizione aminoacidica del micelio e dei liquidi colturali di due ifomiceti, isolati dal suolo, in relazione alla loro nutrizione azotata. Sono state riscontrate differenze quantitative, particolarmente evidenti nel caso di composti azotati la cui trasformazione porti alla formazione di ammoniaca, come urea e cianamide. Rispetto al nitrato sodico, si ha, soprattutto con cianamide, un notevole aumento quantitativo del pool aminoacidico e, in particolare, dell'acido α-aminobutirrico, di cisteina e metionina.

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