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NIVALENOL IN A CULTURE OF AN ISOLATE OF *Fusarium graminearum* 15/2 VNIIVS

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Nivalenol (3,4,7,15-tetrahydroxy-12,13-epoxytrichotech-9-en-8-one), first described as a metabolite of *Fusarium nivale* Fn 2B [1], has recently been identified in a number of isolates of *F. graminearum* and of *Gibberella zaeae* obtained in Japan [2].

In the present paper we report on the isolation of the total fraction of trichothecenes from the biomass obtained after the growth of an isolate of *F. graminearum* Schw. 15/2 VNIIVS on grain and the identification in it of nivalenol by TLC and chromato-mass spectrometry.

The biomass was extracted with a mixture of acetonitrile and water in a ratio of 5:1, and the extract was subjected to preliminary purification by filtration through a column with a two-layer filling of a mixture of activated carbon with Celite and of neutral alumina. The filtrate after concentration in vacuum to an aqueous phase was purified on a "PRE SEP C18" column (Czechoslovakia). The TLC analysis of the fraction isolated on the elution of the column with methanol-water (3:7) on Silufol with chloroform-methanol (7:1), benzene-acetone (1:1), and chloroform-acetone (3:2) as the mobile phases [3], followed by treatment of the plates with a 10% solution of AlCl_3 in ethanol and heating at 92°C for 10 min showed by a blue fluorescence in UV light at λ 366 the presence of substances having R_f values of 0.15, 0.08, and 0.02, respectively, coinciding with the R_f values of an authentic sample of nivalenol. To identify the substance, the dry residue from an eluate was dissolved in a mixture of N,O-bis(trimethylsilyl)acetamide, N-trimethylsilylimidazole, and trimethylchlorosilane in a ratio of 3:3:2, and the solution was heated at 60°C for 20 min and was analyzed on a Finnigan MAT 4615 chromato-mass spectrometer with a capillary column (0.32 mm \times 20 m) containing the stationary phase OV-1 with programming of the temperature from 150 to 280°C at 4°C/min. Mass spectra were recorded under conditions of electron impact (EI) and of chemical ionization by positive and negative ions (CIPI and CINI), the reagent gas being ammonia at 0.7 mm Hg, with an ionizing voltage of 70 V.

The GC analysis of the trimethylsilylation product revealed the presence in it of a main component with retention time of 13.2 min, the EI spectrum of which contained weak peaks of the $(M - 15)^+$ ion (585, 2%) and the peaks of ions with m/z 482, 323, and 242, which are characteristic for the fragmentation of the TMS derivative of nivalenol [4]. In the CIPI mass spectrum, in addition to the peak of the ion of maximum intensity $(M + H)^+$ (601, 100%), there was a fragment $(M + \text{NH}_4)^+$ (618, 55%) and also the peaks of ions with m/z 511 (80%) and 397 (93%). The CINI mass spectrum was characterized by a weak peak of the M^- ion (600, 7%), the peak of a fragment with m/z 297, having the maximum intensity, and the peak of an ion with m/z 303 (20%), and coincided completely with the spectrum of the TMS derivative of nivalenol obtained previously [5].

The amount of nivalenol was 0.004% of the dry biomass. This is the first time that the identification of nivalenol in a culture of the isolate of *F. graminearum* Schw. 15/2 VNIIVS has been reported.

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