Badr [1] reported chromosomal stickiness to be a major abnormality produced by dithane M-45 and denmart in root tip cells of *Allium cepa*. Fragments of both the centric and the acentric type were observed in the present investigation. The acentric fragments originated either as terminal or interstitial deletions of chromosomes, as is clearly indicated by the unequal length of the chromosomes. It is assumed that fragments frequently undergo reunion with the broken ends of the chromatid arms to form various configurations. Acentric fragments were rare, which shows that most of the fragments either undergo fusion, or get lost in the following cell cycles. Nandi [2] reported fragmentation of chromosomes in root tips of *Allium cepa* treated with ceresan and agrosan GN. Similar breaks were also reported in the root tip cells of *Allium cepa* after treatment with denmart and dithane M-45 [1].

From the preceding discussion, it is clear that the fungicide dithane M-45 induced a number of different types of chromosomal aberrations in the bone marrow cells of male mice at the doses tested in the present investigations.

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Chromosome-breaking activity of extracts of the mushroom *Paxillus involutus* Fries ex Batsch

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Summary. Dry and presoaked seeds of *Nigella damascena* were treated with aqueous extracts of the mushroom *Paxillus involutus*. At the first mitosis after the onset of germination, metaphase chromosomes showed damage independent of the origin of the mushrooms. The damaging substance(s) is (are) thermostable. Except a few achromatic gaps, all the lesions observed are of the chromosome type, i.e. are induced at the pre-synthetic G1 stage.
Key words. Chromosome damage; mushroom extracts; *Paxillus*; *Nigella*.

Although some poisonous properties of mushrooms have been known since far into antiquity, the carcinogenic and mutagenic potentialities of some extracts, even those from some edible kinds such as *Agaricus bisporus* or *Gyromitra*, have only recently been suspected [1, 2].

These findings were promptly confirmed for the genus *Lactarius* [3] especially in the commercial pickled species *Lactarius necator* [4]. In this last species, a substance named necatorin showed a high mutagenicity [5]. The occurrence of such mutagens/carcinogens is probably more widespread than was originally expected; for example they exist in several families of Basidiomycetes, and various alteragenic molecules have been already isolated [6].

In these studies, the mutagenicity of extracts was always demonstrated in an Ames' test, using *Salmonella typhimurium* strains with or without S9 mix. This fraction seems to be able to deactivate the mutagens. The fact that clastogenic potentialities have not been investigated prompted new experiments in this field.

Although the species *Paxillus involutus* Fries ex Batsch was considered edible for a long time, more recent, not infrequent cases of poisoning have allowed the identification of a *Paxillus* syndrome, found to be mainly due to a phenol derivative named involutin, the chemical and pharmacological properties of which are now well known [7]. In fact, preliminary experiments with *Paxillus* extracts on plant cells [8] clearly suggested an effect at the chromosome level in higher organisms, and suggested that it would be worthwhile to extend the experiments.

Material and methods

Nigella damascena (var. blue Miss Jekyll) seeds were used to test the clastogenicity of extracts. The special advantages of this plant material for clastogenic studies have been previously described [9]. Mushrooms were harvested in September-October in various habitats avoiding external contaminations such as leaves, fungi, etc., and kept at −20 °C till the beginning of experiments. To prepare extracts, thawed mushrooms were first sliced, then pounded in a mortar in a 0.14 M NaCl sterile solution (at 22 °C), filtered and kept at 4 °C.

To test the thermostability of potential mutagens, extracts were heated in an oven (80 °C/5 h) or evaporated (50 °C for 4 days) before being cooled down to room temperature. After treatment, (100 seeds in 5 ml extract, 5 h at 22 °C), seeds are allowed to germinate under stan-

dardized conditions[9]. Roots are harvested at the first mitotic cycle after the onset of germination (70–72 h), and treated with colchicine (0.05 g%/3 h). After fixation (Carnoy, 2–20 h), slides were prepared from Feulgen squashes.

Results

The results of the experiments are summarized in tables 1–3. Table 1 shows that the effects of *Paxillus* extracts on chromosome damage in dry seeds are amply confirmed.

1) There are significant increases of the damage over the control $\chi^2 = 64.84$, 7df, $p < 0.001$.
2) There are significant differences between samples harvested in the same habitat. Some samples, 1c, 2b and 3a do not show any increase of aberrations over the control $\chi^2 = 4.18$, 3df, $p \simeq 0.25$.
3) There are no significant differences between the habitats in which the mushrooms were harvested $\chi^2 = 4.46$, 3df, $p > 0.20$.

Table 2 shows that for a selected habitat (lawn), there is a significant increase for all presoaking times over the control $\chi^2 = 36.35$, 4df, $p < 0.001$. However, the frequency of aberrations is significantly higher at 40 h presoaking $\chi^2 = 18.98$, 3df, $p < 0.001$, but no significant difference can be detected between dry seeds and 30, 50 h presoaked seeds $\chi^2 = 4.54$, 3df, $p > 0.20$.

Table 3 compares the effects on dry seeds of extracts at room temperature, with those of heated or evaporated extracts from the same origin (lawn) $\chi^2 = 0.53$, 3df, $p \simeq 0.90$.

For all experiments, the frequency of aberrations is significantly higher than the control level $\chi^2 = 19.95$, 3df, $p < 0.001$.

Discussion and conclusion

The present experiments demonstrated the clastogenic activity of aqueous extracts of *Paxillus involutus*. It was also found that this (or these) mutagenic substance(s) is (are) thermostable since heating extracts to 80 °C does not decrease the chromosome damage. This rules out the possibility that one of the clastogenic molecules might be an analogue of involutin, since the latter is thermolabile[7]. The spectrum of chromosome aberrations induced by the extract is quite characteristic. Apart from a

Table 1. Chromosome damage induced by *Paxillus involutus* extracts from various origins (small letters indicate independent experiments) in *Nigella* dry seeds

Habitat		No. of cells analyzed	Chromosome aberrations Breaks (deletions)	Minutes	Gaps	Dicentrics	Rings	Total
1) Birch wood	a	1200	35	4	3	1	0	43
	b	600	8	3	1	0	0	12
	c	400	1	0	0	0	0	1
2) Oak wood	a	200	6	0	1	0	0	7
	b	200	0	1	0	0	0	1
3) Lawn	a	200	3	0	1	0	0	4
	b	200	5	5	1	0	0	11
Control		800	4	1	0	0	0	5

Table 2. Chromosome damage induced in presoaked seeds by *Paxillus* extracts, origin: lawn (200 metaphases analyzed in each case; small letters indicate independent experiments)

Presoaking time (h)		Chromosome aberrations Breaks (deletions)	Minutes	Gaps	Dicentrics	Rings	Total
0	a	5	5	1	0	0	11
	b	3	0	1	0	0	4
	Total	8	5	2	0	0	15
30	a	3	5	0	0	0	8
	b	1	0	0	0	0	1
	Total	4	5	0	0	0	9
40	a	16	7	0	0	0	23
	b	1	0	0	0	0	1
	Total	17	7	0	0	0	24
50	a	1	3	0	0	0	4
	b	1	2	0	0	0	3
	Total	2	5	0	0	0	7
Control	a	1	0	0	0	0	1
	b	1	0	0	0	0	1
	Total	2	0	0	0	0	2

Table 3. Chromosome damage induced by *Paxillus* extracts in *Nigella* dry seeds, origin: lawn (200 metaphases analyzed in each case; small letters indicate independent experiments). 1) Extract at 20 °C; 2) heated; 3) evaporated.

Treatment		Chromosome aberrations					
		Breaks (deletions)	Minutes	Gaps	Dicentrics	Rings	Total
1	a	10	1	0	1	0	12
	b	3	0	1	0	0	4
	Total	13	1	1	1	0	16
2	a	8	1	0	3	0	12
	b	3	1	1	0	0	5
	Total	11	2	1	3	0	17
3		3	2	1	3	1	10
Control		1	0	0	0	0	1

few isochromatid gaps, which can be interpreted as prophasic lesions, all aberrations are of the chromosome type, i.e. induced in the G_1 phase, with a large proportion of chromosome deletions versus interchanges and intrachanges. No aberrations of the chromatid type were observed. The possibility of the accumulation of toxic and mutagenic/carcinogenic substances from parasitic or saprophytic fungi can be generally ruled out by careful examination of the cuticles, and also by the fact that only young carpophores were harvested.

There is also a possibility that the radioactivity accumulated in mushrooms a long time after the Tchernobyl disaster is responsible for the clastogenic effects. The radioactivity was measured several times in various mushroom species, including *Paxillus involutus*[10]. It never exceeded 50×10^3 Bq/kg dry weight for Cs^{134} and Cs^{137}, and 2200 for K^{40}. These levels, although significantly increased, are far too low to explain the effect[9] ($1 \text{ Bq} = 27 \times 10^{-12}$ Ci). The variability of the response within the same habitat raises the problem of a possible metabolic transformation by mushroom tissues of a promutagen into an ultimate mutagen. Up to now, the nature of the substance(s) responsible for the chromosome damage remains unknown, and it should be isolated and identified in further research.

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An evaluation of a somaclone of *Dioscorea floribunda* Mart & Gall

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Summary. 150 plants of *D. floribunda* representing a single clone were regenerated from a stem tissue culture and regenerants were subjected to cytological, phenotypic and biochemical analysis from the pre-transfer stage to three vegetative growth cycles in the field. The plants could be subdivided into three cytological categories, namely, diploid, mosaic and tetraploid. Diploids, mosaics and the one tetraploid showed diversity amongst themselves with respect to internode length, content of chlorophyll and diosgenin. No marked difference in the length and nature of the leaf or in the type of stoma was recorded. Possible causes of the observed variation are discussed.
Key words. D. floribunda; in vitro regeneration; somaclonal variation; diosgenin; mixoploidy.