Cytological Responses of Garlic Root-tip Cells to Some Cigarette Smoke Constituents

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There has been a strong desire among many who are devoted to the problem of tobacco smoke and its effects on human health to perfect a bioassay technique for cigarette smoke and its constituents so as to test their biological effects on living cells and tissues. Tobacco smoke is known to include a number of polycyclic aromatic compounds (Stedman 1968). Some of these are now known to be carcinogenic as determined by mouse skin bioassay technique (Wynder and Hoffman 1967). Nevertheless the vast majority of the chemical constituents of tobacco smoke have not been assayed, largely due to the time and expense involved in mouse skin bioassay procedure (Van Duuren 1975).

In this investigation, the mitotic events in root-tip cells of garlic (*Allium sativum*; family *Liliaceae*) were studied in an attempt to determine the feasibility of utilizing plant material in ascertaining the biological effects of selected cigarette constituents. Earlier work in this laboratory with cigarette smoke (Arnold *et al.* 1976) and smoke constituents, (Bhalla *et al.* 1974), demonstrated induction of mitotic abnormalities in cells of root-tip. The present investigation confirms earlier observations and is of a more comprehensive nature. It deals with the individual and combined effects of 1- and 2-naphthonitrile on cell division and chromosomes of root-tip cells of garlic.

Materials and methods

Commercially available garlic bulbs (*Allium sativum*; family *Liliaceae*) were grown in moist vermiculite at room temperature for 72 hours. At this time, the roots had emerged from the bulbs and were 2-3 centimeters in length. The bulbs were then placed into vials (2 cm × 5 cm) containing various concentrations of 1-naphthonitrile and 2-naphthonitrile, as well as certain combinations of these chemicals for 24 hours. The structural formulae of these compounds are shown in Fig. 1. The concentrations utilized are given in Table 1.

A low concentration of dimethyl sulfoxide (0.5%) was used as a solvent for making stock solutions of 1- and 2-naphthonitrile. The control roots were treated with double distilled water containing dimethyl sulfoxide equivalent to the amount used

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for making solutions of 1- and 2-naphthonitrile. Following treatment, the roots were fixed for 24 hours in a solution of 95% ethanol-acetic acid (3:1) at room temperature, they were then hydrolyzed in 1N HCl at 65°C for about 3 minutes. After hydrolysis, the roots were immediately washed several times with distilled water and stored in acetocarmine until squashes could be made from the apical 2 mm. Twenty-five to thirty slides were made from each treatment. From these, three were finally selected on the basis of well spread cells and high mitotic index for detailed examination. The percentage of cells exhibiting mitotic abnormalities was recorded.

1-Naphthonitrile

2-Naphthonitrile

Fig. 1. Structural formulae of 1-naphthonitrile and 2-naphthonitrile.

Results

In control roots, 93% of the divided cells exhibited normal mitotic divisions. Nuclei of control cells at interphase were essentially spherical. In treated roots, the percentage of mitotic abnormalities was observed to increase significantly in relationship to the concentrations of the compounds utilized. Non-dividing cells of treated roots frequently showed pycnotic and elongated nuclei.

The concentrations utilized for studying the isolated effects of 1- and 2-naphthonitrile were 1, 5, 10, 15, 20, and 25 ppm. Similar concentrations of the two compounds were used for studying their interactions. The most prevalent mitotic abnormalities induced by these chemicals individually and in combination were stickiness, chromosome breakage, chromosome bridging, lagging of individual chromosomes during anaphase, and the formation of micronuclei. However, when interactions of these compounds were studied, scattering of chromosomes, non-synchronization in the movements of chromosomes and polyploid cells were also observed.

The majority of the mitotic abnormalities seen were in metaphase cells. Table 1 summarizes the mitotic abnormalities at metaphase and anaphase in each treatment. From the table it is apparent that the overall effect of 2-naphthonitrile alone is about 4% more pronounced consistently than is 1-naphthonitrile in inducing abnormal mitotic divisions.

When the interactions of 1- and 2-naphthonitrile were investigated, certain promotive effects of induction of mitotic abnormalities became apparent. Probably the most notable of these effects was that only 1 ppm of 1-naphthonitrile was observed to enhance the occurrence of mitotic abnormalities induced by all concentrations of 2-naphthonitrile by as much as 10%. Five ppm of 1-naphthonitrile was also

Table 1. Detailed observations of mitotic abnormalities induced by 1-naphthonitrile (1N) and 2-naphthonitrile (2N) and their interactions.

Treatment	Total dividing cells	Abnormally dividing cells (%)	Mitotic index
1 ppm 1N	119	29.4	0.064
5 ppm 1N	90	32.2	0.054
10 ppm 1N	156	45.5	0.094
15 ppm 1N	144	47.9	0.055
20 ppm 1N	111	45.9	0.048
25 ppm 1N	129	72.1	0.050
1 ppm 1N+1 ppm 2N	186	34.4	0.050
5 ppm 1N+1 ppm 2N	133	44.4	0.049
10 ppm 1N+1 ppm 2N	120	51.7	0.039
15 ppm 1N+1 ppm 2N	89	67.4	0.031
20 ppm 1N+1 ppm 2N	100	68.0	0.036
25 ppm 1N+1 ppm 2N	53	88.7	0.024
1 ppm 1N+5 ppm 2N	170	41.8	0.055
5 ppm 1N+5 ppm 2N	138	53.0	0.055
10 ppm 1N+5 ppm 2N	135	70.4	0.057
15 ppm 1N+5 ppm 2N	111	71.2	0.048
20 ppm 1N+5 ppm 2N	49	81.6	0.021
25 ppm 1N+5 ppm 2N	12	81.7	0.010
1 ppm 2N	165	27.0	0.085
5 ppm 2N	187	39.0	0.085
10 ppm 2N	174	44.2	0.088
15 ppm 2N	139	49.6	0.069
20 ppm 2N	96	58.3	0.054
25 ppm 2N	66	80.3	0.037
1 ppm 2N+1 ppm 1N	186	34.4	0.050
5 ppm 2N+1 ppm 1N	170	41.8	0.055
10 ppm 2N+1 ppm 1N	173	42.8	0.052
15 ppm 2N+1 ppm 1N	135	51.8	0.047
20 ppm 2N+1 ppm 1N	114	65.8	0.035
25 ppm 2N+1 ppm 1N	78	67.9	0.038
1 ppm 2N+5 ppm 1N	138	46.4	0.051
5 ppm 2N+5 ppm 1N	138	55.1	0.055
10 ppm 2N+5 ppm 1N	100	58.0	0.042
15 ppm 2N+5 ppm 1N	66	60.6	0.031
20 ppm 2N+5 ppm 1N	46	80.4	0.021
25 ppm 2N+5 ppm 1N	25	80.0	0.022

found to have a similar effect as 1 ppm 1-naphthonitrile in association with 2-naphthonitrile. The overall effect of 1 ppm of 2-naphthonitrile in presence with different concentrations of 1-naphthonitrile appeared less pronounced. These treatments had about 5% more mitotic abnormalities than did corresponding concentrations of 1-naphthonitrile. Five ppm of 2-naphthonitrile enhanced the overall effects of the different 1-naphthonitrile treatments by about 11%. The detailed effects of these interactions are shown in Fig. 2. The mitotic index, in general,

declined when the roots were treated with higher concentrations of either 1- or 2-naphthonitrile in isolation or in combination.

Fig. 2. Effect of various concentrations of 1- and 2-naphthonitrile alone and in combination at various levels on the mitotic abnormalities in the root-tips of garlic.

Discussion

The present investigation demonstrates that 1-naphthonitrile and 2-naphthonitrile are quite capable of inducing mitotic abnormalities in dividing garlic cells. These observations are in general conformity with earlier work from this laboratory (Bhalla *et al.* 1974) and elsewhere (Izard *et al.* 1970). The present work was extended basically for noting possible interactions of 1- and 2-naphthonitrile. Low concentrations of either of these two analogues appear quite capable of enhancing the overall effects of the other on mitotic divisions of garlic cells.

Observations of meristematic plant cells are quite simple for the determination of the percent of mitotic abnormalities as well as the mitotic index. These two calculations may give the observer a quick, easy, and accurate indication of the relative overall potency of the compounds tested. These same parameters, however, would not necessarily indicate the relative carcinogenicity of these compounds to animal systems. Nevertheless, a bioassay technique such as the one employed in this investigation could conceivably be of value in ascertaining the tobacco smoke constituents which are most toxic to living cells. Presently, the mouse skin-painting technique (Van Duuren 1975) is the most commonly used bioassay for determining the relative carcinogenicity of cigarette smoke constituents. It is known to be expensive and time-consuming. All of the observations of this investigation were made within two weeks following the initial time when the garlic bulbs were allowed

to germinate. Thus, the possibility of using plant material as a simple and efficient bioassay of tobacco smoke constituents should be further investigated. Such an assay might be of value in screening large numbers of samples rapidly and accurately for research. By this means, certain of the more potent chemical components of tobacco smoke might be determined and removed from tobacco before it is used for commercial products.

Summary

Root-tips of garlic (*Allium sativum*) were used to study the cytological effects of 1-naphthionitrile and 2-naphthionitrile in isolation and in combination. The treated interphase cells showed pycnotic and elongated nuclei. The mitotic abnormalities observed in treated root-tip cells included scattering of chromosomes, non-synchronization in the movements of chromosomes, stickiness, chromosome breakage, bridges during anaphase, lagging of whole chromosomes, and the formation of micronuclei. Low concentrations of one compound appear capable of enhancing the overall effects of the other with respect to inducing mitotic abnormalities. The mitotic index was observed to decrease with increasing concentrations of either or both of these two constituents. It is suggested that this bioassay technique might be more economically feasible for preliminary rapid screening of the biological effects of tobacco smoke constituents from various cigarettes.

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Literature Cited

- Arnold, R. C., Bhalla, P. R. and Sabharwal, P. S. 1976. Cytological responses of *Allium sativum* to smoke puffs from various types of cigarettes. *Cytologia* **41**: 543-551.
- Bhalla, P. R., Arnold, R. C. and Sabharwal, P. S. 1974. Cytological investigations on onion root-tip cells treated with some cigarette smoke constituents. *J. Hered.* **65**: 311-313.
- Izard, C., Valadaud, D. and Morée-Testa 1970. Sur l'activité chromatoclasique et mitoclasique de certaines fractions de goudrons de fumée de cigarette. *C. R. Acad. Science. Paris* **270**: 2457-2459.
- Stedman, R. L. 1968. The chemical composition of tobacco and tobacco smoke. *Chem. Rev.* **68**: 153-207.
- Van Duuren, B. L., Sivak, A., Katz, C., Seidman, I. and Melchoinne, S. 1975. The effect of aging and interval between primary and secondary treatment in two-stage carcinogenesis on mouse skin. *Can. Res.* **35** (3): 506-511.
- Wynder, E. L. and Hoffman, D. 1967. Tobacco and Tobacco Smoke. Academic Press, New York, New York.
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