

WHEAT COLEOPTILE ELONGATION TEST FOR BIOASSAY OF HOMOEOPATHIC DRUGS

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Abstract

A simple, quick and highly sensitive technique is proposed for the bioassay of homoeopathic drugs. Young wheat coleoptile segments have shown responses to the potency for centesimal dilutions of homoeopathic drugs viz., *Arnica montana*, *Aconitum napellus*, *Cimicifuga racemosa*, *Dioscoria villosa*, *Hypericum perforatum*, *Ignatia amara*, *Lemna minor*, *Nux vomica* and *Pulsatilla nigricans* within six hours of treatment.

Introduction

In homoeopathic drug system the action of a drug is not due to the mass but the fine division of the medicinal substance. Plant responses to different homoeopathic drugs have been studied by Pelikan & Unger (1971) and Jones & Jenkins (1981). The present paper describes a simple and quick technique for evaluating a number of aqueous homoeopathic drugs upto a very high dilution of 10^{-42} .

Material and methods

Growth of young coleoptiles of wheat was tested according to the method of Wright (1961). Seeds of *Triticum aestivum* cv. Pak-81 were soaked in distilled water for 2 h, and placed on moist filter paper in Petri dish with their embryos facing upward. The dishes were incubated at $25 \pm 1^{\circ}\text{C}$ in dark for 24 h. Under a low power binocular microscope (20 x), young coleoptile apical segments were excised using the epiblast as the guide (Fig.1). The excised coleoptiles were washed in water in a beaker and 10 coleoptiles placed with their enclosed primary leaves on to a microscopic slide. A drop of water was added and the coleoptiles measured under a binocular microscope (20 x) with the help of a ocular micrometer. Each group of 10 coleoptiles was then placed on a 3 cm x 1 cm strip of Whatman No.1 filter paper which was inserted into a 14 x 2.5 cm test tube containing 0.3 ml of test solution. Three replicates were used for each treatment. Test tubes were covered with black sheet and incubated at 25°C . After 6 and 20 hours of incubation, length of coleoptiles were again measured and the mean increase in length calculated. Two way classification was used for the analysis of variance.

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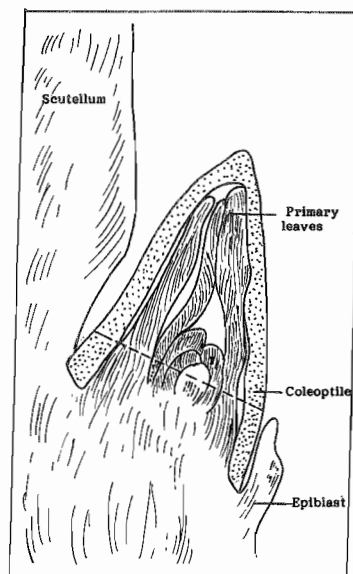


Fig.1. Excision of young wheat coleoptile.

Method of Potentization: Distilled water was used as the diluting medium throughout the present study. Mother tinctures (Φ) of *Arnica montana*, *Aconitum napellus*, *Dioscoria villosa*, *Pulsatilla nigricans*, *Nux vomica*, *Lemna minor*, *Ignatia amara*, *Cimicifuga racemosa* and *Hypericum perforatum* were obtained from Willmar Schwabe of West Germany. Potencies were freshly prepared as centesimal aqueous dilutions by employing Eylea metabolic shaker, model NTS-311. Each sample was given, at each stage, 80 sharp impacts in 100 ml glass bottle containing 10 ml of the homoeopathic drug. At interval of 5 minutes, the next potency was prepared in the centesimal scale from third to twenty first centesimal dilution (10^{-6} - 10^{-42})

Results

The young coleoptile of wheat excised 24 h after sowing has been found to give a good growth response to the aqueous homoeopathic drugs. The results presented in Figs. 2-4 gave an indication of the promotion of wheat coleoptile growth by all the nine drugs used. The result of the two way classification of analysis of variance showed that all the treatments of remedies were significant while in between the potencies of *Aconitum*, *Hypericum* and *Pulsatilla* were non-significant (Table 1). After 6 h of incubation in the dark, no increase in the length of control coleoptiles was found, whereas the coleoptiles treated with different homoeopathic drugs showed significant growth promotion (Table 1). When the data of various aqueous dilutions was plotted according to the attenuated dilution on one axis in most cases sinusoidal fluctuation was observed between the maxima and minima. This sinusoidal curve frequently deviated from control data at about 6-7 centesimal dilutions (Figs. 2-4).

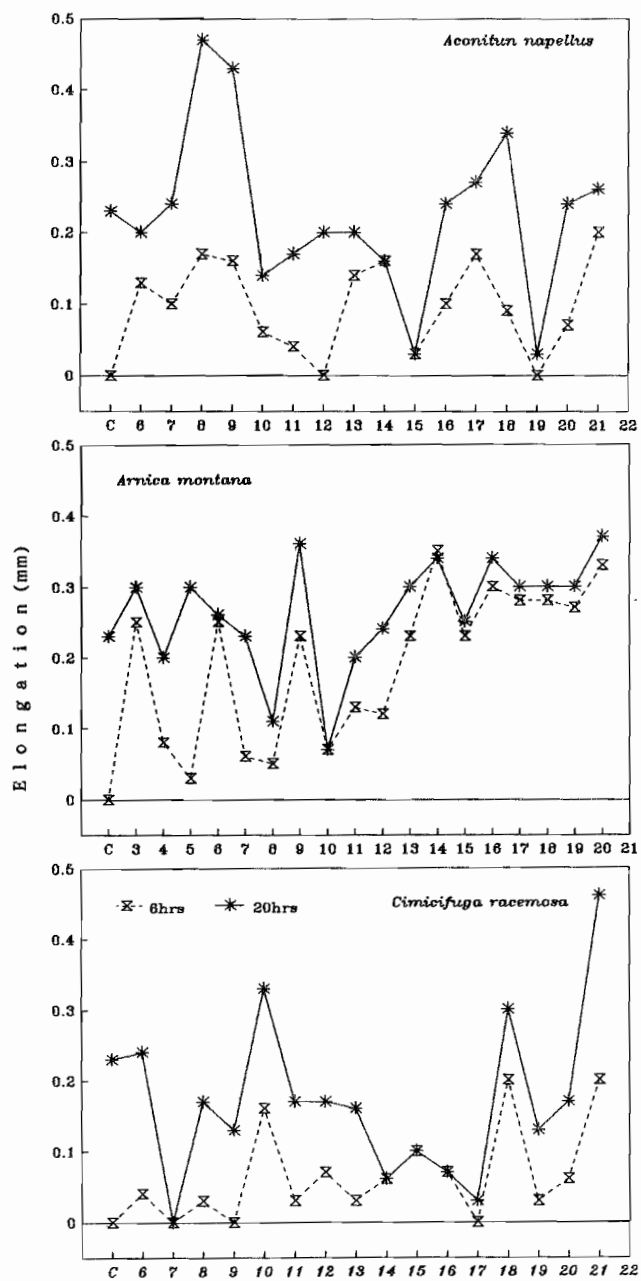


Fig.2. Growth of young coleoptiles segment of wheat in response to the potency for centesimal dilution of *Aconitum napellus*, *Arnica montana* and *Cimicifuga racemosa*.

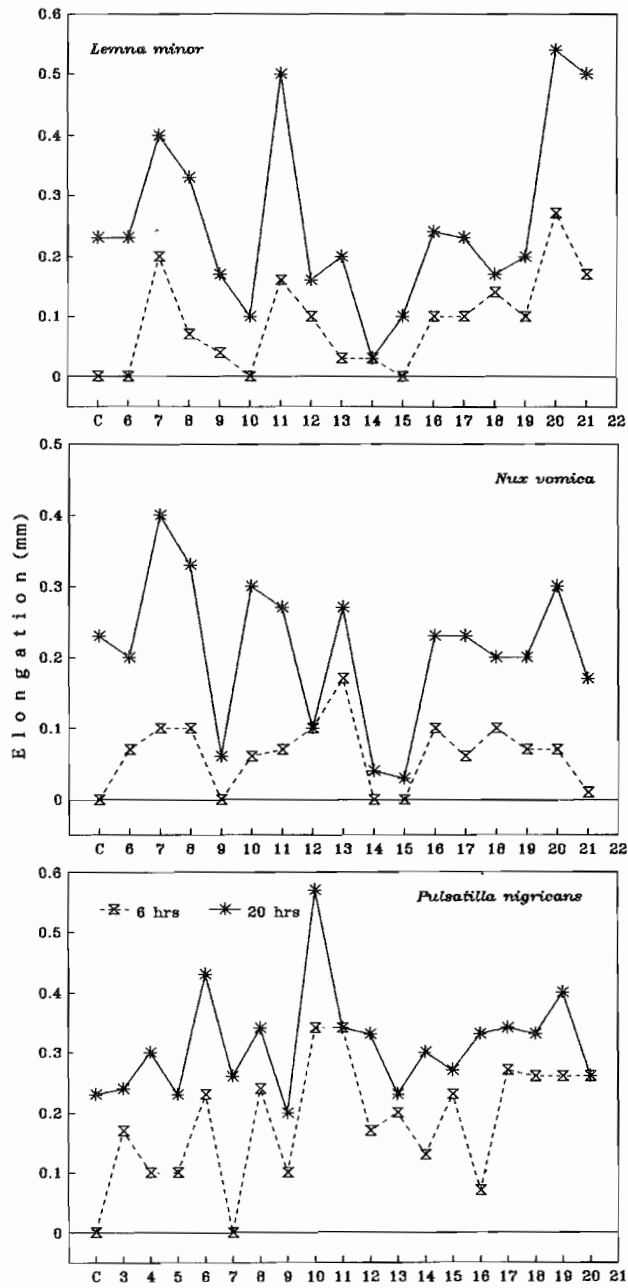


Fig.3. Growth of young coleoptiles segment of wheat in response to the potency for centesimal dilution of *Lemna minor*, *Nux vomica* and *Pulsatilla nigricans*.

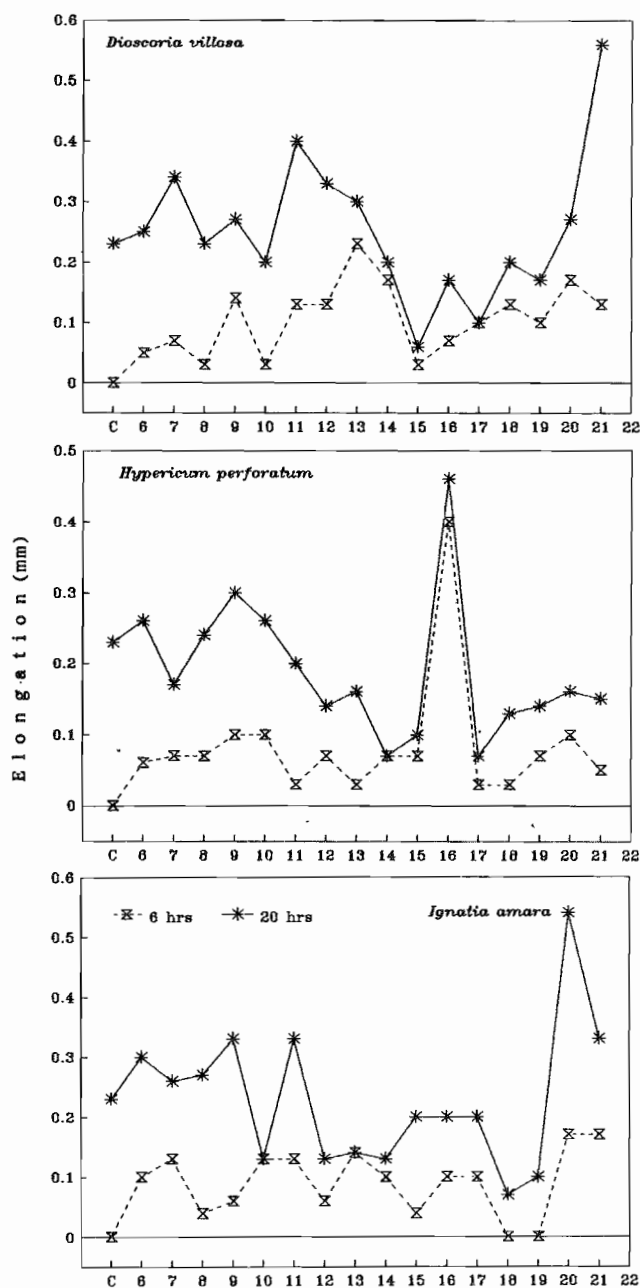


Fig.4. Growth of young coleoptiles segment of wheat in response to the potency for centesimal dilution of *Dioscoria villosa*, *Hypericum perforatum* and *Ignatia amara*

Table 1. *F* values for different homoeopathic drugs and their treatments on young wheat coleoptile.

	Between Potencies	Between Treatments
<i>Arnica montana</i>	10.3895 ^{**}	25.718 ^{**}
<i>Aconitum napellus</i>	0.2221 ^{ns}	21.1733 ^{**}
<i>Cimicifuga racemosa</i>	4.0575 [*]	133.0545 ^{**}
<i>Dioscoria villosa</i>	8.219 ^{**}	85.045 ^{**}
<i>Hypericum perforatum</i>	0.3206 ^{ns}	53.1889 ^{**}
<i>Ignatia amara</i>	6.295 ^{**}	104.454 ^{**}
<i>Lemna minor</i>	5.591 ^{**}	86.924 ^{**}
<i>Nux vomica</i>	7.0409 ^{**}	68.5884 ^{**}
<i>Pulsatilla nigricans</i>	2.398 ^{ns}	2.6078 [*]

* Significant at 5% level ** Significant at 1% level ns: Non-significant

Discussion

The growth pattern of all the 9 drugs follow a typical sinusoidal curve as was also observed by Jones & Jenkins (1981) and Baker & Smith (1985) with homoeopathic medicines. According to Schrödinger (1961) atomistic disorder in the bio-system could be corrected by imparting order from the stereo-specific regularity of the solvent polymer. The structural informational content of a chemical or bio-organ-chemical agents may exhibit a significant biological role with respect to its associated chemical mass. It is therefore possible that a solute may provide a base for polymerization of the solvent in the presence of solvent molecules as observed in the present study.

When substances are diluted beyond 10^{-24} , the original substances do not exist in the solution, still the biological activity of the substance could be demonstrated at much higher dilutions as was evident from the present study and by the starch diastase experiment of Boyd (1942, 1946, 1954). It may be mentioned that the presence of a physical factor in very high dilutions (beyond 10^{-24}) has already been worked out by Gay (1951, 1952), Boyd (1954), Saify *et al.*, (1976), and Benveniste (1988).

Pelikan & Unger (1971) and Jones & Jenkins (1981) have shown that the growth of wheat seedlings could be used in monitoring the effect of homoeopathic drugs. The growth of intact wheat coleoptile was found to be altered after 6 days of contact with the homoeopathic drugs (Jones & Jenkins, 1981). However in the present study it has been shown that the excised wheat coleoptile growth is altered within 6 h of contact with the homoeopathic drugs. Thus the modified procedure proposed in the present study could be utilized in analyzing the aqueous homoeopathic drugs within a very short span of 6 h.

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(Received for publication 30 April 1991)