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Evaluation of Genotoxic Potential of Vanilla Essence on Mitotic Chromosomes of Hordeum Vulgare

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Abstract: ***Vanilla essence is often used as flavoring in food might be attained by adding vanilla essence or by cooking vanilla pods in the liquid preparation. The conc. used of this essence is very low because of its poisonous effect in higher conc. An experimental data analyzed that the vanilla essence has toxic effect on mitosis chromosomes of Hordeum vulgare. Four conc. 0.1ml, 0.2ml, 0.3ml and 0.4ml were used for the treatment of root tip of test plant. There were number of abnormalities observed like stickiness of chromosomes, unequal distribution of chromatids, scattered chromosomes, chromatin bridges, un-oriented and unequal distribution of chromosomes etc. These types of abnormalities were increased with increased conc. and duration of treatment of this essence.***

Keywords: Chromosomal aberrations, Genotoxic, Vanilla essence

1. Introduction

A main exercise of vanilla is in flavoring ice cream. Even though vanilla is an award winning flavoring agent on its own, it is also used to get better taste of new materials, to which its own taste is repeatedly balancing, such as custard, chocolate, coffee, caramel, cakes, and others. This species is among multiplicity of vines recognized in profusion in Mexico. This plant grows best indoors all along with other houseplant. Though marketable farming of vanilla is done outdoor, Hernan Cortes, the well recognized guide, was the initial to find out this vine in Mexico and bear the vanilla plant to Europe. Vanilla is a perfumed tonic. This herb is supposed to refresh the mind, supplement the energy of the muscles, turn away sleep and encourage the sexual capability. Additionally, vanilla is broadly used in arranging scents, and adding quintessence to confectionery, tinctures, creams, syrups and so on. Traditional health check texts comprise state about vanilla.

Fascinatingly, at the same time as vanilla is itself a valued essence agent, the herb is also made use of in attracting the strength of other substances where the taste of vanilla is repeatedly that of a balancing agent. Custard, chocolates, coffee, caramel and other substances are example of this feature.

Many workers have studied the outcome of some chemicals on the chromosomes structure and their presentation throughout cell division. Some of the important work had been done by Asahina et al., (1976), Strurelid et al. (1972), Swami et al. (1976), studies the extort of Lichens effects on the mitotic division in Allium cepa and Allium sativum. The extracts of Lichens recognized to reveal antibiotic, fungistitic and mitrostatic properties. Cytokinin (BA) endorse cell division which is still happening in fruits is useful for thinning. Root tip of Allium cepa treated with 50 and 100ppm of BA for 3 h, 6 h, 12 h showed different mitotic irregularity such as anaphase, bridges, laggards, multipolar spindles formation, C-mitosis, micronuclei and endo reproduction (Yang and Soh, 1993).

2. Material and Method

To determine the toxicity of vanilla essence on Hordeum vulgare, the factor included various conc. 10%, 20%, 30%, 40%, 50% for root tip treatment of Hordeum vulgare. First of all the seeds of Hordeum vulgare were washed methodically and soaked in distilled water for 4-7 min, after that the seeds were germinated on damp filter paper in sterilized petriplates. When the root tips were 2.2mm long were subjected into 0.1ml, 0.2ml, 0.3ml and 0.4ml solution of Orchis mascula for 8, 12, and 24 hrs. After treatment root tips were rinsed with distilled water and fixed in carnoy's solution (GAA and Ethyl alcohol) for 6-10 hrs followed by and transfer into 70% of alcohol. Slides have been prepared by acetocarmine squash method. The observations were taken under 100X with oil emersion. Some of the important stages were photographed by using Samsung E5 phone.

3. Observations

There were numerous structural, behavioral and numerical aberrations were observed in microslides of *Hordeum vulgare* by vanilla. Those were as follows:

Mitotic Index

The mitotic index was reduced with increase in concentration (0.1-0.4ml) and period (4-24hrs) of treatment. The mean percentage value of mitotic index was drop off from 6.38 to 5.83% (Table-1).

Sticky, Scattered, Swollen and Unequal distribution of chromosomes

The treatment of vanilla resulted in stickiness, swollenness of chromosomes at metaphasic plate (Fig-A, B, C, and D). The aberrations were noticed in increasing order of duration and conc. The mean percentage value was higher from 6.06 to 6.27% in *Hordeum vulgare* (Table-1).

Multipolar spindle and inactivation

Multipolar spindle were observed in *Hordeum vulgare*. The aberration increased with increased in conc. and period of treatment. The mean percentage was 0.12 to 0.05% (Table1).

Chromatin bridges, Sticky chromatids, Unequal distribution of chromatids

Vanilla essence induced chromatin bridges, sticky chromatids and unequal distribution of chromatin formation in *Hordeum vulgare* were also observed in higher conc. (Fig- E, F, and G).

Figures showing an assortment of chromosomal aberrations during different stages of mitotic cell division in root tip cells of *Hordeum vulgare.*

4. Results and Discussion

Results revealed that vanilla essence has potential to damage chromosomes and its content. Such changes vary stimulation and/or reduction of mitotic index, formation of a great number of chromosomal aberrations was appeared by the treatment of vanilla on Hordeum vulgare. The variations in the percentage of each different types of chromosomal aberration noticed in this study was dosage independent (Soh and Yang, 1993 et al.,). D. Amato (1949, 1952) suggested that reduced MI by the chemical mutagens has resulted due to prophase poisoning and restriction of the separating cells of prophase or interphase stage. In the present investigation it was clearly showed that appearance of sticky, scattered chromosomes was the most prominent abnormality observed (Table-1).

Low concentrations of tobacco leaf extract exerted a stimulating effect, whereas high concentration acted as a mitodepressant, on root-tip cells of Allium sativum L. (Sopova et al., 1983). This abnormality was also explained by Swaminathan and Nataranjan (1956) on the foundation of breakdown of cytokinesis and destruction of spindles apparatus or storage inhibition of spindles formation. Extracts of leaves and inflorescences of male spinach and aster plants increased the frequency of chromosomal aberrations and mutations in Welsh onion and barley, respectively, whereas the female plants inhibited the processes (Sidorskii, 1984). The chromosome movement is unfavourably pretentious by Freg-Wyssling, 1938. Epel (1963) noticed the complete inhibition mitosis when ATP level dropped the 50% of normal level.

The presence of a cytokinin-like substance in the extract has been suggested to be responsible (Krivokapic et al., 1970). Abraham and Cherian (1978) investigated the cellular changes produced by extracts of betel leaves on root tip cells of onion and demonstrated the cytotoxicity of such extracts.Chromosome-breaking activity has been exhibited by aqueous extract of mushroom (Paxillus involutus) in dry and presoaked seeds of Nigella damascena L. (Gilot-delhalle et al., 1991).

Chromatin bridges were also observed due to occurrence of chromatin stickiness and subsequent failure of anaphase separation or it may be the result of chromosome breaks ad reunion (Grover and Virk 1986). According to the Sharma (1985) fragments followed by translocation may guide to heritable phenotypic difference.

The micro nuclei and multi nuclei were observed in low incidence. Micronuclei are true mutagenic effect and these are a strong correlation between the capabilities of a fragrances or chemical to induce chromosomal aberrations and chromosomes fragments (Torkoglu et al., 2007). Micronuclei are true mutagenic effect (Kirkla D. 1998).

5. Conclusion

On the basis of observations and results our findings show that vanilla essence evaluated have a genotoxic potential with increased in concentrations and duration of treatment. Such evaluation should include other endpoints of genotoxicity. The inhibitory action tends to lower the active dose of genotoxic agents and the accelerating action raises it.

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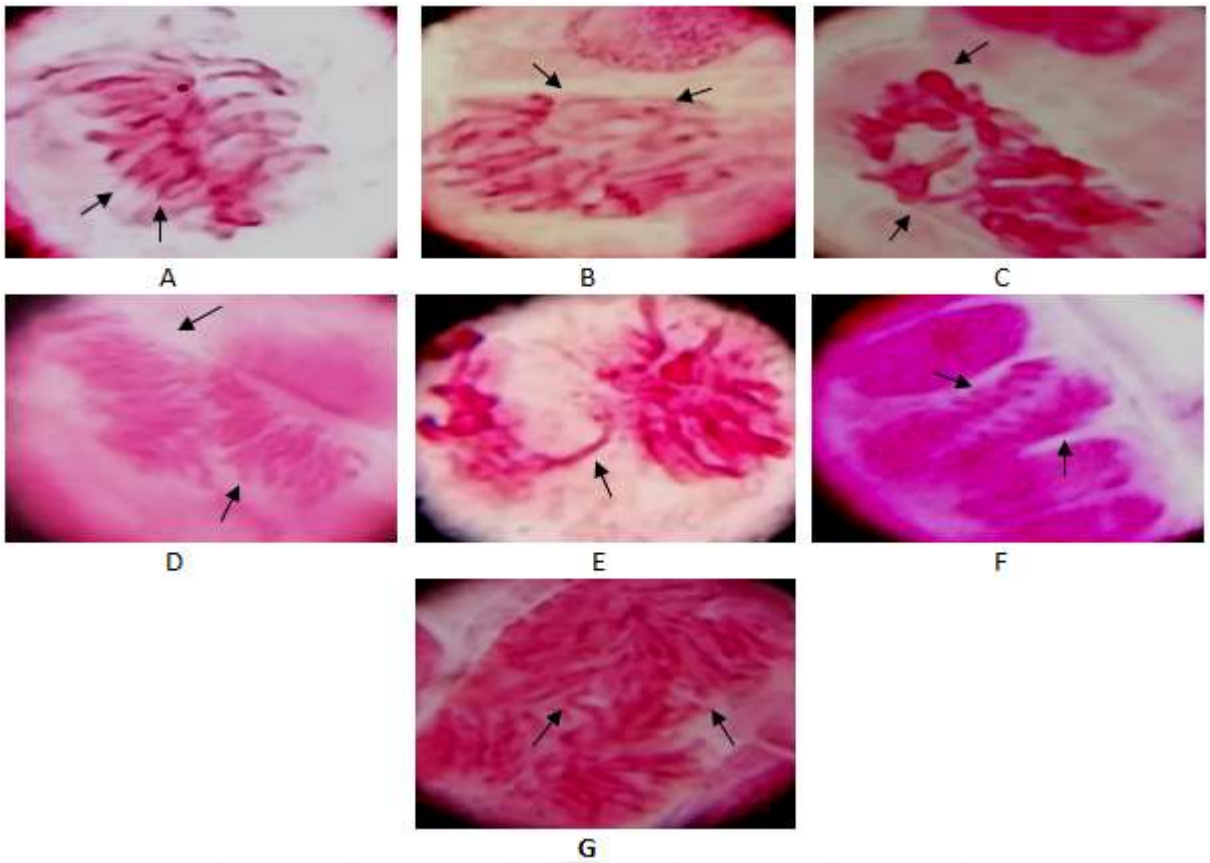

Figure: A-Metaphase with sticky chromosomes. B- Metaphase with scattered chromosomes. C- Metaphase with swollen chromosomes. D- Metaphase with un-oriented and unequal distribution of chromosomes. E- Anaphase with single chromatin bridge. F- Anaphase with sticky chromatids. G- Anaphase with unequal distribution of chromatids.

Table 1: Type and distribution of Somatic Chromosomal abnormalities (%) induced by different concentrations and duration of Vanilla essence (Root-tip treatment) in *H. vulgare*

Duration	Conc. in %age	Total Cell Observed	Mitotic Index	Condensed and Sticky Chromosomes	Multi Polar spindles	Fragments	Chromatin bridges	Multi nucleate Cells	Laggards	Polyploidy	Total % of abnormalities
4 hrs	Control	315	10.78	0.28	-	-	-	-	-	-	0.25
	0.1ml	290	6.21	5.90	-	-	-	-	-	-	5.85
	0.2ml	288	5.90	6.19	-	-	-	-	-	-	6.19
	0.3ml	260	5.25	6.95	-	-	-	-	-	-	6.88
	0.4ml	258	5.18	7.98	0.15	-	-	-	-	-	9.10
	0.5ml	225	5.00	9.10	0.60	-	1.60	0.60	-	-	11.95
	Mean	272.6	6.38	6.06	0.12	-	0.26	0.10	-	-	6.70
8 hrs	Control	215	10.55	0.29	-	-	-	-	-	-	0.20
	0.1ml	291	5.98	6.16	-	-	-	-	-	-	6.18
	0.2ml	284	5.62	6.75	-	-	-	-	-	-	6.68
	0.3ml	245	4.89	7.49	-	-	-	-	-	-	8.39
	0.4ml	230	4.45	8.59	0.20	-	-	0.70	-	-	10.35
	0.5ml	221	4.26	9.90	0.90	-	1.70	0.90	-	-	13.50
	Mean	264.3	5.98	6.53	0.18	-	0.28	0.26	-	-	7.55
12 hrs	Control	316	10.65	0.18	-	-	-	-	-	-	0.16
	0.1ml	292	5.81	5.95	-	-	-	-	-	-	6.95
	0.2ml	287	5.70	7.12	-	-	-	-	-	-	7.10
	0.3ml	260	5.00	8.80	-	-	-	-	-	-	8.55
	0.4ml	252	4.45	9.15	0.70	-	-	0.85	-	-	10.48
	0.5ml	241	4.75	10.09	0.90	-	1.85	0.95	-	-	14.50
	Mean	274.6	5.89	6.76	0.26	-	0.30	0.30	-		7.95
24 hrs	Control	320	10.75	0.21	-	-	-	-	-	-	0.20
	0.1ml	292	5.90	6.13	-	-	-	-	-	-	6.10
	0.2ml	272	5.75	6.19	-	-	-	-	-	-	6.18
	0.3ml	260	4.20	7.19	-	-	-	-	-	-	7.19
	0.4ml	264	4.01	8.25	0.10	-	-	0.50	-	-	7.54
	0.5ml	246	4.39	9.68	0.25	-	1.50	0.25	-	-	11.39
	Mean	275.6	5.83	6.27	0.05	-	0.25	0.08	-	-	6.43

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