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(RESEARCH ARTICLE)

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Phytochemical profiling with cytotoxic and genotoxic potential of green tobacco leaf extract

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Abstract

Tobacco (genus Nicotiana), primarily cultivated for commercial use, poses significant occupational health risks, particularly during its green leaf stage. This study investigates the cytotoxic and genotoxic effects of green tobacco (Nicotiana tabacum L.) through phytochemical analysis using GC-MS and cytological assays on Allium cepa. GC-MS results revealed major compounds including nicotine (42.55%), naphthalene (34.08%), neophytadiene, phytol, and various methyl esters. In cytological duration dependent exposure of root tip meristematic cell and young flower buds in green leaf extracts showed reduced mitotic index as well as meiotic index and different types of chromosomal abnormalities such as stickiness, bridges, laggards, and micronuclei formation.

Keywords: GC/MS;Green tobacco leaves; Genotoxicity; Cytotoxicity; Pulverization

1. Introduction

Tobacco belonging to genus *Nicotiana* is the universally cultivated non- food cash crop. The genus is considered to be originated in South America, several species of Australia and South pacific Islands are known as derivatives of the South America. In India tobacco was introduced by Portuguese in 1605. It belongs to family Solanaceae. There are about 70 recognized species of *Nicotiana,* but two species *Nicotiana tabacum* L.and*Nicotiana rustica* L. are commonly cultivated for producing commercial tobacco. India, having diverse climatic condition which supports the cultivation of both the species of *Nicotiana* in different regions*.* Northern and north- eastern areas of the country i.e. Uttar Pradesh, West Bengal Bihar and Assam are dominated by *Nicotiana rustica* as it requires cooler climate. *Nicotiana tabacum* is cultivated in the region of Gujarat, Andhra Pradesh, north Bihar and some area of Bengal. The N. *tabacum* also known as desi plants are taller than *N. rustica* and have broad leaves with pink flower whereas *N. rustica* is also known as "vilayati and calcuttia and short plants have round puckered leaves with yellow flower. Out of 70 recognized species of *Nicotinana,* 45 species are maintained in India. 0.45million hectare land is engaged in tobacco cultivation in India i.e.0. 27 % of the net cultivated land producing 750 M kg of tobacco leaf. Its cultivation is providing livelihood security to around 36 million people that include 6 million farmers, 20 million farm labour and 10 million people who are engaged in tobacco processing, manufacturing and export sector (ICAR- CTRI[1]). As India is the second largest producer of tobacco in the world after China and country is also second largest exporter of unprocessed tobacco. Thus tobacco exports contribute sizable foreign exchange to the Indian exchequer. There is an increase in export growth by 87% during the past 5 years i.e. it achieved a record high export value of Rs. 12,005.89 crore in the year 2023-24 as against Rs. 6,408.15 crore in 2019-20. During this period the export volume have increased from 218.84 million Kg to 315.51 million Kg (Ministry of Commerce and Industry).

Besides harmful usage of cured tobacco leaves in smoking and chewing green leaf was known for its medicinal values. In India and China, raw green tobacco leaves were used for treating painful piles, rheumatic swelling (Agyare 2013[19]).

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Since ancient times these plants are used by Peruvian Amazonian community for treating mental health, parasitic illness and respiratory problems, especially *Nicotiana rustica* L. is described as master plant in this region. The green tobacco preparation was administered in either liquid or solid form via oral, tropical intranasal and rectal note. Although there are many reporting about the neuropsychiatric disorders caused by green tobacco leaves but still it is used in native place Peruvian Amazon because there was a notion that green tobacco having spiritual energetic property (Berlowitz*et.al.*2020[20]). On small scale green tobacco extracts is used by local farmers as green pesticides to kill pests like aphids, jassids and whiteflies. The high nicotine content is also having insecticidal properties (Gudeta*et. al.* 2021[21]).

Tobacco farming, although economically important, has considerable health and social implications, especially for agricultural workers and labours engaged in harvesting, curing, and processing the leaves. One of the most critical health issues among tobacco workers is Green Tobacco Sickness (GTS). It is as toxic as cured form i.e. smokeless tobacco (khaini) and cigarette.Few research papers are focused on occupational anthropogenic hazard of green tobacco leaves responsible for Green tobacco sickness (GTS) prevalent in Asian and South American tobacco harvesters. The sickness occurs due to the intradermal absorption of nicotine from the wet surface of tobacco plants. GTS symptoms include nausea, vomiting, dizziness, delirium, increased perspiration, abdominal pain, diarrhea, increased salivation, weakness, breathlessness and occasional lowering of blood pressure (SHAILEE F et.al 2017[22]). Vomiting can lead to dehydration and adds to the risk of heat illness. These symptoms are so common that GTS remains ignored and so not well documented. Tobacco is grown in more than 100 countries and tobacco processing is all done manually by the labourer so there is extended exposure of GTS which may lead to disease like cardiovascular or may have mutagenic effect which manifested as cancer (Mc Bride *et.al.*1998[23]). The prevalence of GTS among workers varies between 8% to 47%, with increased susceptibility among women, adolescents, and individuals working without protective gear.

In this study phytochemicals analysis of fresh green tobacco leaf has been done through GC/MS and genotoxicity and cytotoxicity were assessed by analyzing chromosomal aberrations in meiotic and mitotic cells of *Allium cepa* flower bud as well as meristematic cells of root tip respectively.

2. Material and methods

2.1. Sample collection

Fresh green tobacco leaves were grown from seeds procured from Indian Agricultural Research Institute Pusa, Bihar.

2.2. Extract Preparation for GC/MS Analysis

Fresh green tobacco leaves were dried in shade and powdered.For extract preparation 100 gm of dried leaf powder was extracted with 500ml of methanol using an orbital shaker for 72 hours. The extraction process was repeated with the same solvent until the solvent became clear and colourless. The resulting extract was then evaporated for drying and stored in an airtight container at 4 °C for future use.

2.3. GC/MS Analysis

Methanol extract of green tobacco leaves was sent to AIRF, JNU, New Delhi for GC-MS analysis. GC-MS analysis was carried out in Shimadzu QP-2010 Plus with Thermal Desorption System TD 20. The carrier gas used was Helium at the flow rate of 16.3 ml/min and column flow rate of 1.21 ml/min. The amount of sample used was 6µl, and the mode of injection was split with a temperature of 260 ºC. The column oven temperature was set at 100 ºC. The total running time of GC-MS was 40 minutes. For determining the phytochemicals, the obtained retention time and mass weight were compared with the GC-MS spectra database of online Wiley library and NIST (National Institute of Standard and Technology).

2.4. Extract preparation for Cytological study

For extract preparation 100grams of green leaves were thoroughly crushed with 1000ml double distilled water and left for 10 hours and then filtered through whatman filter paper 5.

2.5. *Allium cepa* assay

Allium cepa seeds were soaked in double distilled water for 9 to 10 hr. Soaked seeds were kept on moist filter paper for germination in flat dishes. Immediately after germination germinated seeds were exposed to green tobacco leaves extract for 15, 30, 45and 60 minutes. After extract exposure root tips were transferred in carnoy's fixative (1:3 acetic acid: alcohol) for 24 h and were preserved in 70% ethanol. Some unexposed germinated seeds were directly fixed by transferring in carnoy's fixative and preserved in 70% alcohol which serves as negative control. The exposed tips as

well as negative control tips were separately heated in acetocarmine for staining and slides were prepared by squash and smear technique and were observed under Magnus s/n: C197050239 microscope. Photographs were taken at 40X.

To determine the effect of the leaves extract, mitotic index (MI) were calculated for different time period as: -

$$\text{MI} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells observed in one focus}} \times 100$$

Abnormality percentage (AB %) was calculated by following formula.

$$\text{AB \%} = \frac{\text{Total number of abnormal cells}}{\text{Total number of dividing cells in one focus}} \times 100$$

3. Results

The GC-MS analysis of green tobacco leaves revealed the presence of several major compounds (Table 1 and Figure 1) such as nicotine, naphthalene, 1,2,3,6-tetrahydro-2,3′-bipyridine, neophytadiene, phytol, stigmasterol, β-sitosterol, hexadecanoic acid methyl ester, methyl stearate, and 9,12-octadecadienoic acid methyl ester includingsclareolide, palmitic acid derivatives, and various long-chain hydrocarbons and esters.

Microscopic examination of exposed meristematic root tips cells and young flower buds of *Allium cepa* showed gradually decreasing number of dividing mitotic cells as well as dividing meiocytes of bud as compared to control. Different types of chromosomal abnormalities were observed in different phases of cell divisions. The increase in abnormalities was dependent on the exposure time period as mentioned in Tables 2 and 3. The entire experiment was conducted in five replicates. A broad spectrum of clastogenic, aneugenic and non-clastogenic aberrations were observed in mitotic cells of root tips as well as flower bud exposed to green tobacco leaves extract. The clastogenic abnormalities like stickiness (Fig.7,8,9,16,17), laggards (Fig. 8, 12, 19) and non-clastogenic like disorientation at metaphase, anaphase and telophase (Fig.11, 18)were observed at 15 min, 30 min, 45 min and 60 min of exposure time. While micronuclei (Fig. 20), nuclear budding (Fig. 13), abnormal Sporades like triads (Fig 22), disoriented Sporades (Fig 23) were observed only at high exposure time i.e. 60 minutes. Multiple bridges were observed at 30 and 45 minute exposure time (Fig. 10). The study also showed that mitotic and meiotic abnormalities were directly proportional to the exposure time (Figure 4 and 6).

Table 1 Compounds identified in methanolic extract of green tobacco leaves (GL) by GC/MS

PeakReportTIC				
Peak#	**R.Time**	**Area**	**Area%**	**Name**
1	7.096	9608896	34.00	NAPHTHALENE
2	8.270	143907	0.51	Sulfurousacid,2-ethylhexylisohexylester
3	9.153	218939	0.77	4-(DIMETHOXYMETHYL)-1,2-DIMETHYLBENZENE
4	9.351	12024327	42.55	PYRIDINE,3-(1-METHYL-2-PYRROLIDINYL)-,(S)-
5	11.063	106355	0.38	2-Buten-1-ol,2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl
6	11.144	126991	0.45	Sulfurousacid,2-ethylhexylhexylester
7	11.590	227002	0.80	1,2,3,6-Tetrahydro-2,3'-bipyridine
8	13.667	135320	0.48	Heneicosane
9	15.148	520184	1.84	Neophytadiene
10	15.601	137873	0.49	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
11	16.044	1537843	5.44	Hexadecanoicacid,methylester
12	17.677	102526	0.36	9,12-Octadecadienoicacid,methylester
13	17.738	472748	1.67	8,11,14-Docosatrienoicacid, methylester
14	17.842	620504	2.20	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-,[R-[

15	17.979	917719	3.25	Methylstearate
16	18.378	118181	0.42	4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-
17	18.752	286025	1.01	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-,pivalate
18	20.663	40928	0.14	2-Methoxydecanoicacid
19	21.090	130936	0.46	Glycerol,2-TMS-
20	21.894	63432	0.22	1,2-Cyclohexanedicarboxylicacid,bis(2-ethylhexyl)ester
21	22.913	51424	0.18	Per-O-trimethylsilyl-(3-O-.beta.-d-mannopyranosyl-d-gluci
22	26.065	97789	0.35	7-(2-HYDROXY-1-METHYLETHYL)-1,4A-DIMETHYL
23	27.153	83569	0.30	Stigmasterol
24	27.448	330176	1.17	Stigmasterol
25	28.195	157161	0.56	.beta.-Sitosterol
		28260755	100.00	

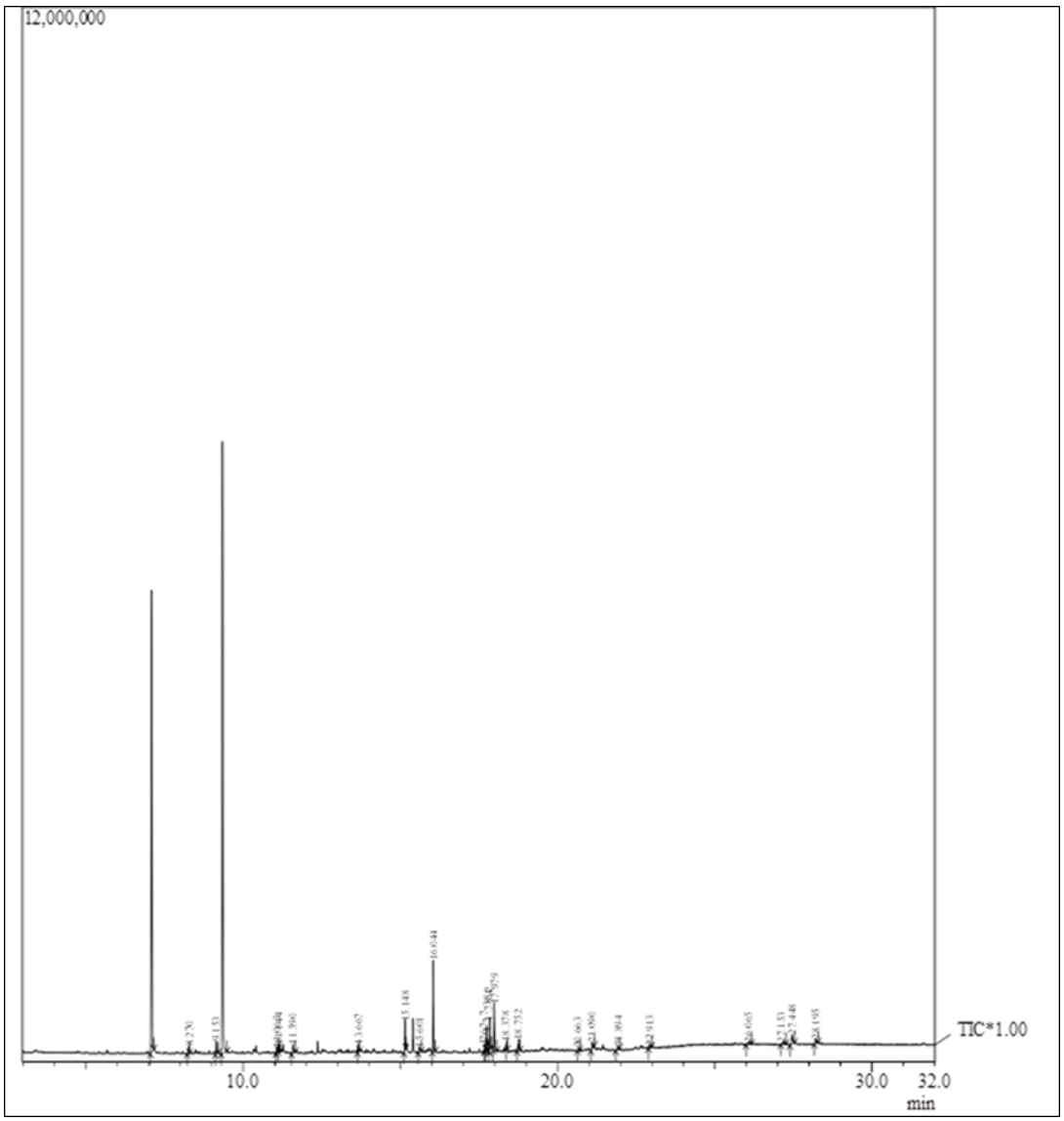


Figure 1 GC-MS Chromatogram of methanol extract of green tobacco leaves

Table 2 Effect of green tobacco leaf extract on mitotic cells of root tip of *Allium cepa*

Duration	**control**	**15 minutes**	**30 minutes**	**45 minutes**	**60 minutes**
Total number of cell observed	2500	2500	2500	2500	2500
Total number of dividing cell	566	500	453	380	300
Total number of abnormal cells	0	38	47	62	70
Mitotic index (%)± S.E	22.64 ±2.86	20.00± 2.07	18.1 ±2.19	15.2±2.0	12.0 ±1.7
Sticky metaphase	-	1.7 ±0.7	1.8 ±0.75	2.8 ±1.01	3.68 ±1.1
Bridge	-	0.9 ±0.63	1.05 ±0.62	1.64 ±0.49	2.36 ±0.4
Laggard	-	0.96 ±0.64	1.2 ±0.41	1.88 ±0.47	2.63 ±0.62
Micronuclei	-	0.7 ±0.41	0.84 ±0.43	1.64 ±0.44	2.36 ±0.44
Pulverized cell	-	0.7 ±0.45	1.05 ±0.1	1.4 ±0.74	1.31 ±0.61
Disorientation	-	0.5 ±0.49	0.84 ±0.44	1.64 ±0.43	1.81 ±0.60
Blebs	-	0.5 ±045	1.05 ±0.1	1.1 ±0.63	1.81 ±0.44
Other abnormalities	-	0.7 ±0.42	1.05 ±0.1	1.1 ±0.61	1.31 ±0.65
Abnormality (%)± S.E	-	7.6 ± 1.8	10.37 ±1.07	16.31± 4.2	23.33± 1.17

Table 3 Effect of green tobacco leaf extract on meiocytes of bud of *Allium cepa*

Duration	**Control**	**15 minutes**	**30 minutes**	**45 minutes**	**60 minutes**
Total number of cell observed	1750	1750	1750	1750	1750
Total number of dividing cell	1415	1370	1335	1310	1255
Meiotic Index% ± S.E.	80.85± 5.07	78.2 ±5.8	76.2 ±4.5	74.8 ±5.2	71.7 ±3.4
Diakinesis/ Diplotene	-	-	0.15 ±0.52	0.23 ±0.4	0.3 ±0.43
Metaphase-I- Stickiness, Disorientation	-	-	0.35 ± 0.41	0.5 ±0.48	0.6 ±0.37
Anaphase I- Bridge, Laggard	-	-	0.33 ± 0.45	0.38 ±0.45	0.55 ± 0.32
Telophase I- Bridge, Laggard	-		0.29 ±0.58	0.39 ±0.41	0.5 ±0.3
Anaphase I, Telophase –I- Micronuclei, Disorientation	-	-	0.22 ± 0.47	0.52±0.37	0.6 ±0.32
Metaphase II- Stickiness, Disorientation	--	-	0.29 ±0.41	0.44 ±0.51	0.56 ±0.36
Anaphase II- Bridge, Laggard	-	-	0.30 ±0.51	0.35 ±0.44	0.3 ±0.52
Telophase II- Bridge, Laggard	-	-	0.3 ±0.64	0.15 ±0.69	0.15 ±0.51
Anaphase II, Telophase II- Micronuclei, Disorientation	-	-	0.14 ±0.54	0.35 ±0.56	0.45 ±0.34
Meiotic Products (triad, Pentad Hexad)	-	-	0.15 ±0.66	0.22 ±0.41	0.35 ±0.64
Sterile Pollen	-	-	-	0.07 ±0.69	0.23±0.58
Other Abnormality					
Abnormality % ± S.E	-	-	3.5±0.78	4.2 ±0.49	5.09 ±1.1

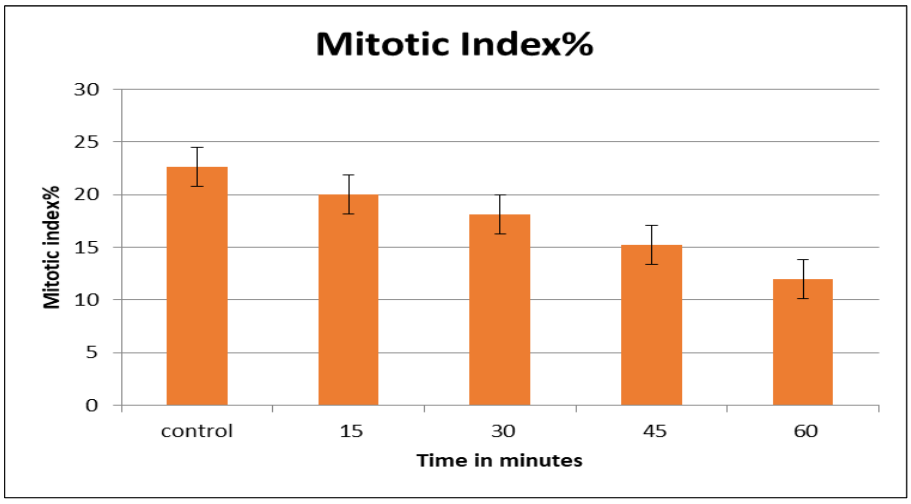


Figure 2 Effect of green tobacco (*Nicotiana tabaccum*) leaves extract : Mitotic Index % of *Allium cepa* L

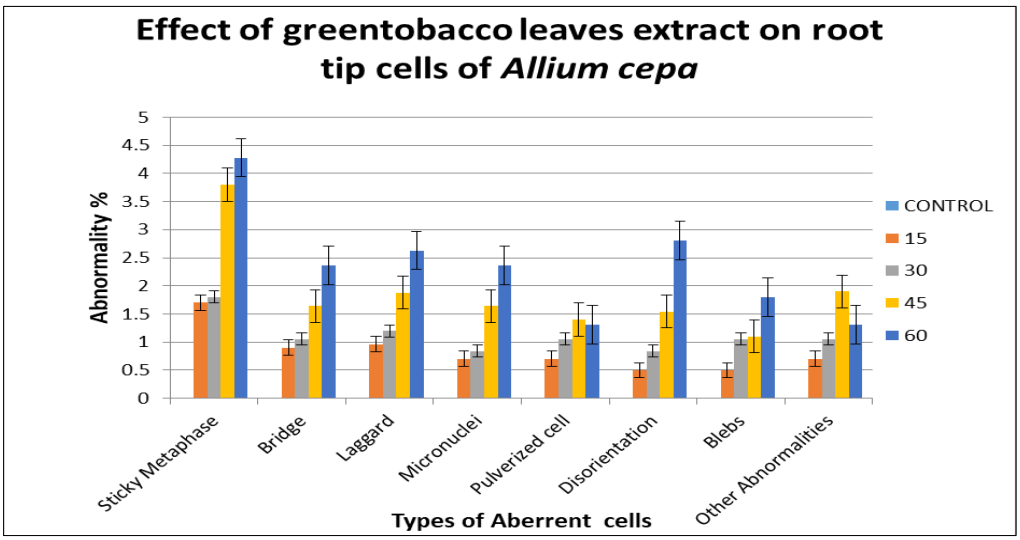


Figure 3 Effect of green tobacco (*Nicotiana tabaccum*) leaves extract on mitotic cells of *Allium cepa* L

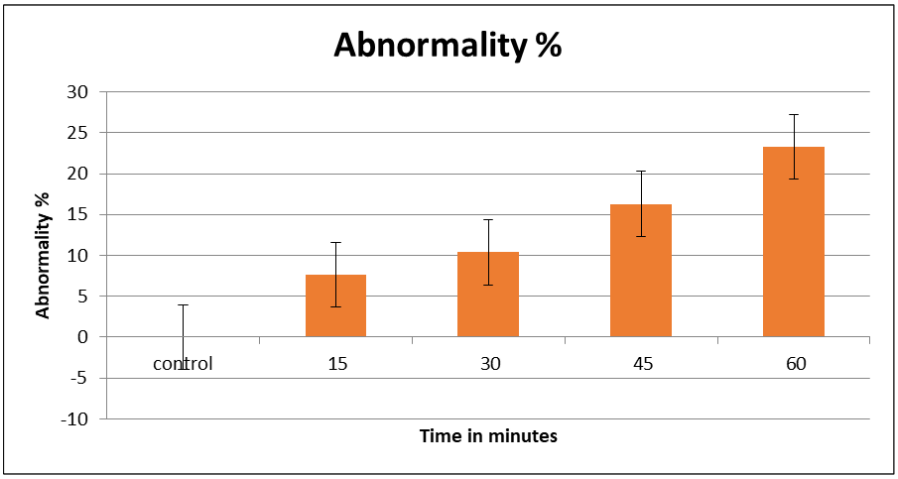


Figure 4 Effect of green tobacco (*Nicotiana tabaccum*) leaves extract : Abnormality % of *Allium cepa*L.mitotic cells

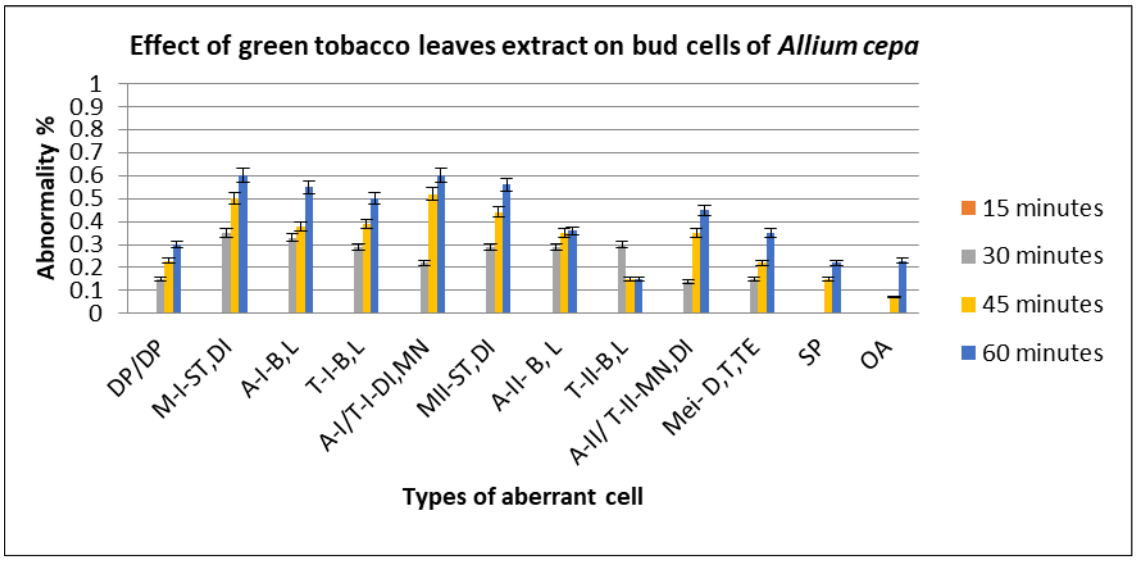


Figure 5 Effect of green tobacco (*Nicotiana tabaccum*) leaves extract on bud cells of *Allium cepa*

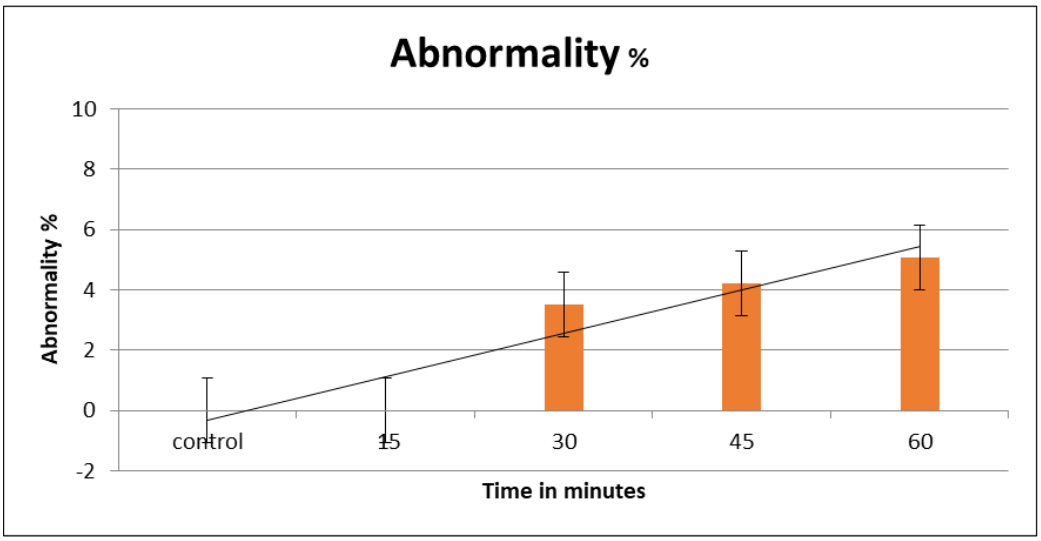


Figure 6 Effect of green tobacco (*Nicotiana tabaccum*) leaves extract on bud cells of *Allium cepa* L. (Column) : Abnormality

3.1. Photograph showing effect of green tobacco leaf extract on mitotic cells of root tip of *Allium cepa*

Figure 7 Ring at sticky Prophase

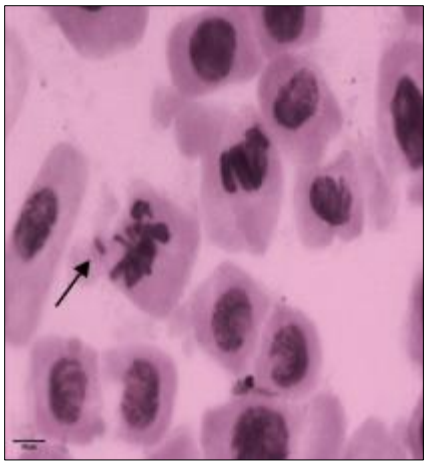


Figure 8 Laggard and Stickiness at Metaphase

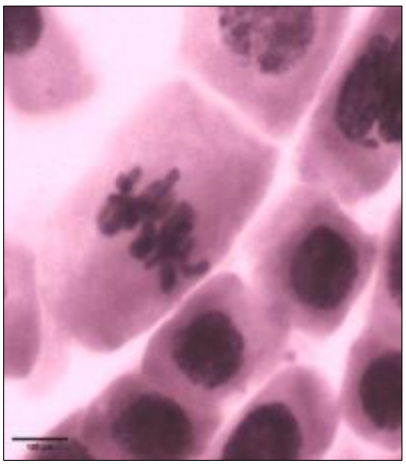


Figure 9 Sticky Metaphase

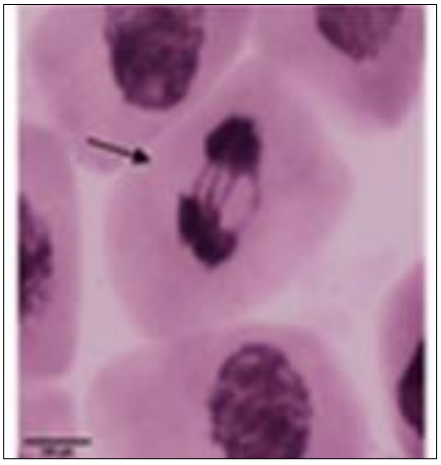


Figure 10 Multiple Bridges at Anaphase

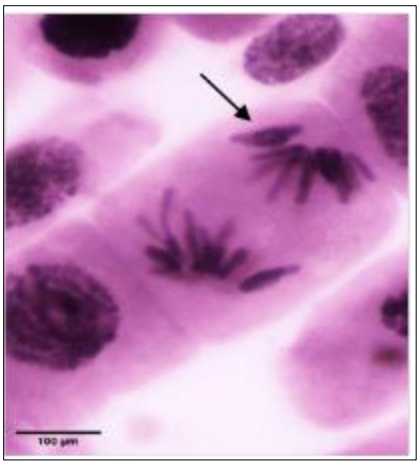


Figure 11 Diagonal Anaphase

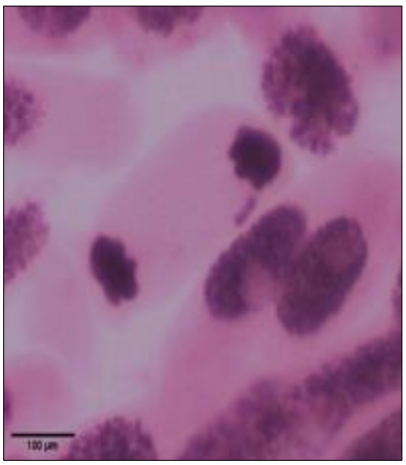


Figure 12 Laggards at Telophase

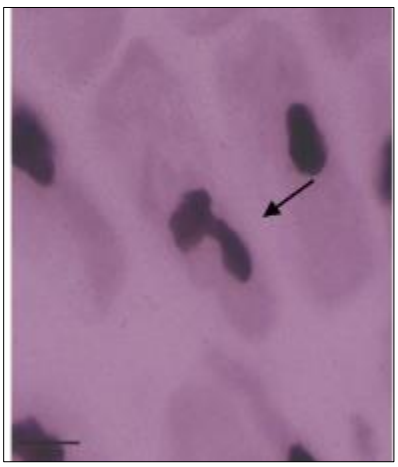


Figure 13 Nuclear budding

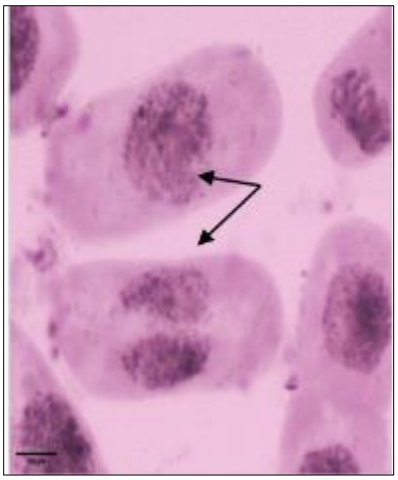


Figure 14 Nuclear erosion

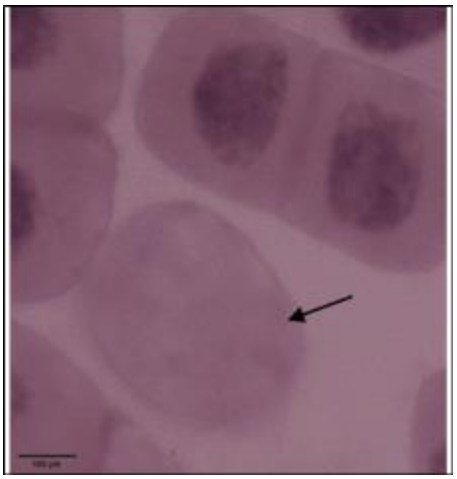
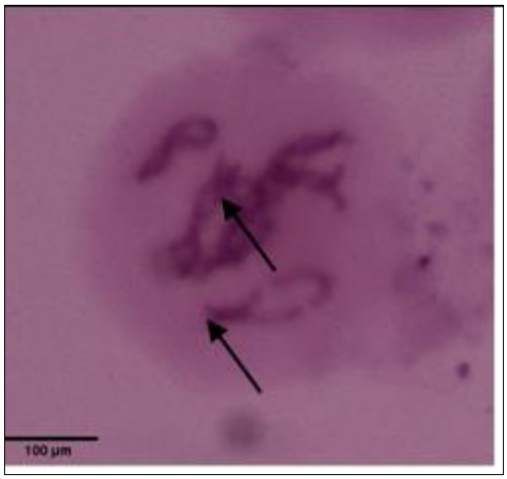


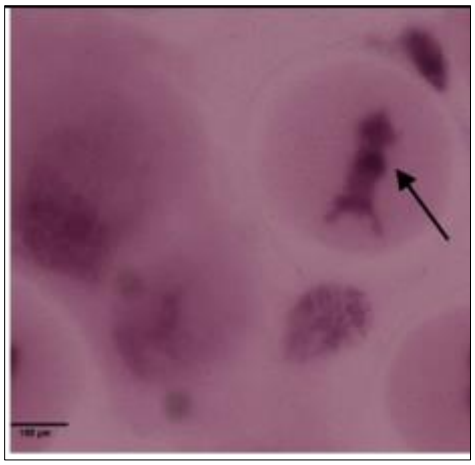
Figure 15 Ghost cell

3.2. Photograph showing effect of green tobacco leaf extract on meiocytes of bud of *Allium cepa*



Photographs are taken under 40X Magnification; scale bar: 100µm

Figure 16 Stickiness and pulverization at Diplotene



Photographs are taken under 40X Magnification; scale bar: 100µm

Figure 17 Stickiness at Metaphase I

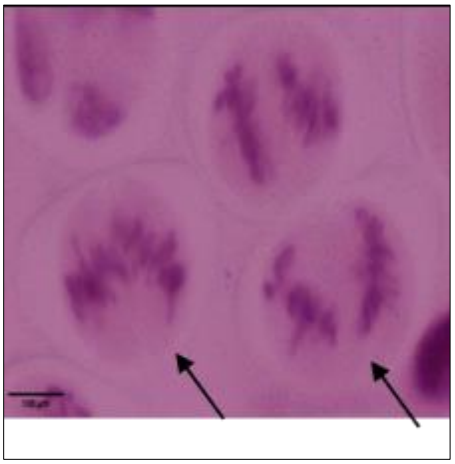


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Figure 18 Disorientation at Metaphase II

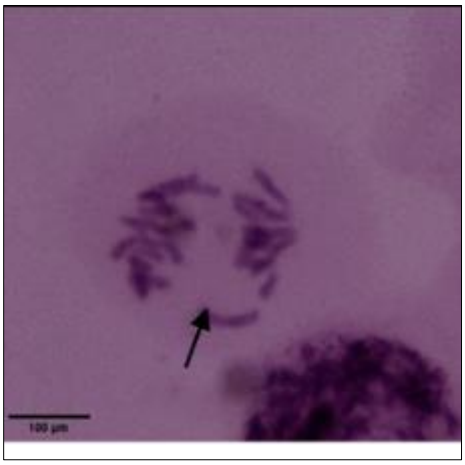

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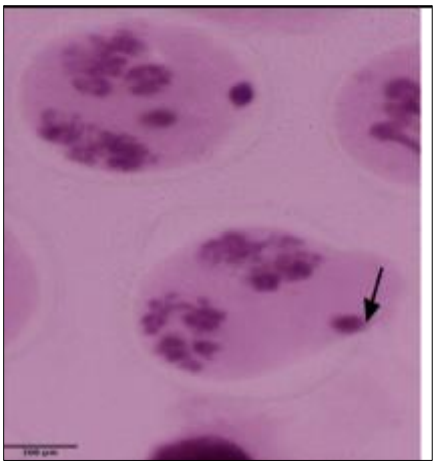
Figure 19 Chromosomal laggard at Anaphase I



Photographs are taken under 40X Magnification; scale bar: 100µm

Figure 20 Pulverized Anaphase I with micronuclei

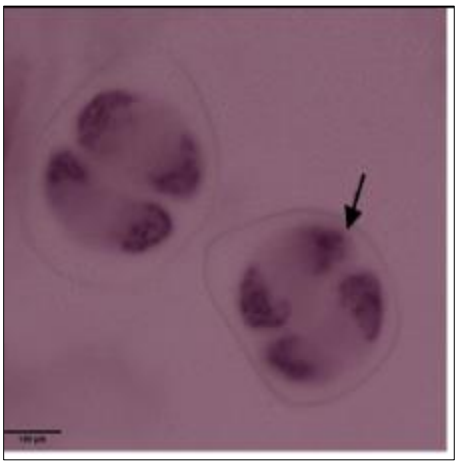


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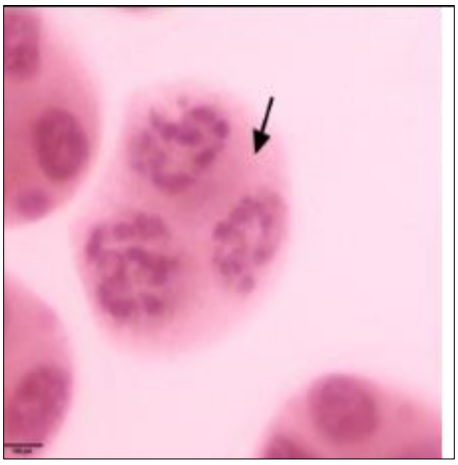
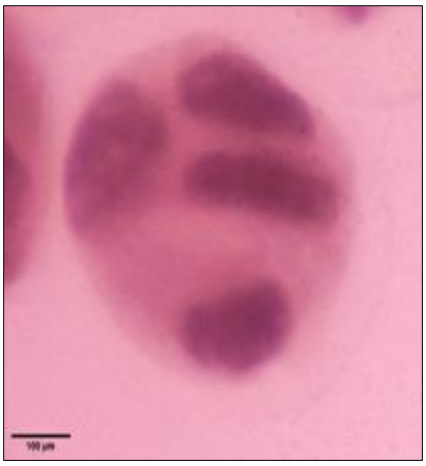
Figure 21 Chromatin erosion at telophase II



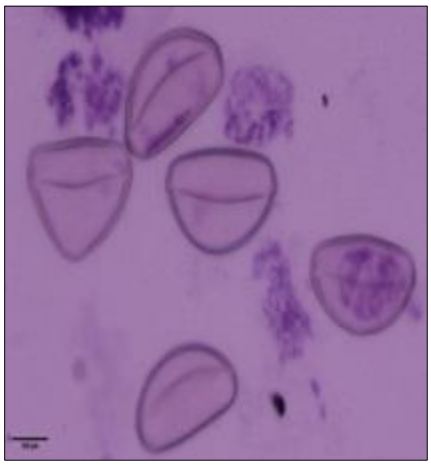
Photographs are taken under 40X Magnification; scale bar: 100µm

Figure 22 Pulverized chromosomes at triad



Photographs are taken under 40X Magnification; scale bar: 100µm

Figure 23 Disorientation at tetrad



Photographs are taken under 40X Magnification; scale bar: 100µm

Figure 24 Deformed Sterile pollen

4. Discussion

Tobacco farming exposes agricultural workers to a variety of chemical constituents, both naturally occurring and introduced through processing. The GC-MS analysis of green tobacco leaves provides insight into the range of volatile and semi-volatile organic compounds which includes nicotine, naphthalene, 1,2,3,6-tetrahydro-2,3′-bipyridine, neophytadiene, phytol, stigmasterol, β-sitosterol, hexadecanoic acid methyl ester, methyl stearate, and 9,12-octadecadienoic acid methyl ester. Some of these chemicals like nicotine; naphthalene can pose serious health risks, especially when exposure is chronic and unprotected. Cytological study on Allium cepa cells that indicates that green tobacco leaves extract induces genetic damage on chromosomes of mitotic as well as meiotic cells of Allium cepa chromosomes. Research from multiple studies, including those conducted in Brazil, has confirmed a high prevalence of GTS, especially among women, with rates exceeding 50% in some populations (Da Mota e Silva MS2018, Alves J 2020) . Nicotine, the principal alkaloid in tobacco, was found to be present at a concentration of 42.55% in the present study .While plucking leaves from field labourers are getting long time intradermal exposure of nicotine from leaves. That may be the reason; previous researchers have shown increased DNA damage at molecular level in tobacco farmers. As nicotine is a precursor of different types of TSNAs (Tobacco specific nitrosamines) , a potent carcinogen when get entered inside the cell causes epigenetic changes. These changes play an important role in the development of some cancer by silencing a tumour suppressor gene as well as the genes that help in repair damaged DNA, leading to an increase in DNA damage which in turn increases cancer risk (Stephen B et. al.2023).The alkaloid especially nicotine is associated with oxidative damage which results in DNA lesion. The study revealed that there is increase in DNA damage from 22 % to 36% in workers with GTS symptoms in comparison to non exposed workers. (Alves J 2020). Tobacco farmers also experiences a substantial exposure to TSNA which is a major carcinogen found in raw as well as cured tobacco leave. It occurs naturally in green tobacco leaves and their concentration increases in considerable amount

during processing and curing. (A.W. Caliri et. al 2021). Beyond acute symptoms, chronic exposure to nicotine and associated compounds has been linked to genotoxic and cytotoxic effects in exposed individuals. Studies using assays like the Comet assay and micronucleus test in tobacco farmers' lymphocytes have revealed significantly elevated DNA damage, shortened telomeres, and altered methylation patterns—indicators of genomic instability, oxidative stress, and premature cellular aging [Da Silva FR 2012, Kahl VFS2018).

The GC/ MS study observed a notably high concentration of naphthalene, accounting for approximately 34.08%, which underscores the environmental relevance of polycyclic aromatic hydrocarbons (PAHs). PAHs are composed of multiple aromatic (benzene) rings bonded with carbon and hydrogen atoms. These structures can form either linear or branched configurations.Studies have demonstrated that naphthalene and its derivatives can induce chromosomal aberrations in plant cells, indicating their genotoxic and cytotoxicpotential. For instance, research on Allium cepa root meristematic cells exposed to naphthalene acetic acid (NAA), a synthetic auxin derived from naphthalene, revealed several cytogenetic abnormalities, including c-mitosis, chromosomal bridges, stickiness, and lagging chromosomes—suggesting both clastogenic and aneugenic effects. Notably, a concentration of 5 ppm NAA resulted in the highest percentage of irregular cell shapes and a significant decrease in the mitotic index, reflecting inhibited DNA synthesis and impaired cell division [Abou-Zeid HM et.al.2020].

Additionally, studies on 1-naphthaleneacetamide (NAAm), another naphthalene-derived plant growth regulator, have shown chromosomal aberrations in human peripheral blood lymphocytes. Although this pertains to human cells, it supports the genotoxic potential of naphthalene derivatives across biological systems [Yilmaz S et.al. 2016].

Further reinforcing these findings, a 2024 study investigated the effects of naphthalene on *Vicia faba* (broad bean) plants and observed significant cytogenetic disruptions. Exposure to naphthalene concentrations ranges from 1 to 7 g/L induced cytomixis—a process involving the transfer of nuclear material between pollen mother cells (PMCs) via cytoplasmic channels or direct fusion. This phenomenon was accompanied by various chromosomal abnormalities, including stickiness, laggards, bridges, uneven chromosomal separation, micronuclei formation, and chromosomal loss. The study also reported a reduction in the meiotic index, increased formation of abnormal sporads (such as triads, dyads, monads, and polyads), and a significant rise in pollen sterility and seed abortion rates at higher naphthalene concentrations. These results underscore the genotoxic effects of naphthalene on plant reproductive cells and highlight the potential ecological risks associated with its environmental presence [Khatab HA 2024].

Chromosomal aberration like stickiness was most prominent and observed in all time periods. These mitotic as well as meiotic chromosome stickiness is may be due to the mutation in recessive st gene which results in chromosome aggulination, reported for the first time in maize (beadle G W) or structural change in the specific non- histone proteins (topoisomerase II and peripheral proteins) which are required for proper segregation of chromatids (Pessim C et al 2015). It was also observed that stickiness was correlated with frequency of bridges formation. This can be supported by the fact that stickiness may results from the improper folding of chromosomal fibre which allows chromatids connection with sub chromatid bridges (Klasterska I et al. 1976, McGill et. al. 1974). Chromosomal bridges were also commonly observed in both mitotic and meiotic cells during anaphase and telophase. As stickiness is very prominent at all the stages this could be one of the major reasons for the occurrence of bridges. Unequal translocation or inversion of chromosome segments results in the formation of dicentric chromosomes could also be the reason of bridge formation (Gomurgen 2005). Lagging chromosomes fragments also known as laggards were frequently observed. This type of physiological aberration arises due to the failure of the attachment of acentric fragment of a chromosome to the spindle fibre (Kumar V et. al. 2015). These Laggard leads to the formation of micronuclei.

Micronuclei (MN) are a biomarker of the genotoxic effect of the extracts that results in genetic instability. MN are small nuclei like structure that is formed by chromosome fragments or whole chromosomes incorporated in the nuclear envelope and persists into interphase due to failing to be reincorporated into primary nucleus after completion of cell division.(Kurpina K et. al. 2021).

This study also observed chromosome pulverization at various stages including diplotene, triad. Chromosome pulverization may result from premature chromosomal compaction (PCC), driven by mitotic cyclin-dependent kinase activity, which induces the condensation of partially replicated chromosomes. (Crasta, K et.al 2012).

5. Conclusion

The current finding is in agreement with above research. GC-MS analysis of green tobacco samples supports these findings, identifying high levels of pyridine derivatives and polycyclic aromatic hydrocarbons like naphthalene—compounds with known mutagenic and carcinogenic potential. The present study has shown that exposure of such

chemicals induces chromosomal abnormalities and abnormal tetrad and spores as well as reduction in mitotic and meiotic index in plant models like *Allium cepa*, demonstrating the cytotoxic and genotoxic nature of green tobacco extracts. These overlapping results from human and plant models underline the pressing need for preventive occupational health measures, including the use of personal protective equipment, monitoring of redox biomarkers, and broader policy interventions to mitigate long-term health risks among tobacco farming communities.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no financial support associated with this work.The authors declare there is no conflict of interest.

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