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Afforestation of post-fire *Pinus brutia* Ten. forests: Effects of *Eucalyptus camaldulensis* Dehnh., *P. brutia* and *Pinus pinea* L. leaf extracts on cell division in *P. brutia* seeds

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ABSTRACT

We investigated the allelopathic potential of *Eucalyptus camaldulensis* Dehnh., *Pinus pinea* L. and *Pinus brutia* Ten. leaf extracts [(0 (control), 25, 50 and 100%)] on cell division and chromosome structure during the seed germination and seedling development of *P. brutia*.. The effects on *P. brutia* mitotic index, phase indices and genotoxicity index were scored and statistically interpreted. The results showed that these species used in afforestation of post-fire had positive and negative effects on the growth of *P. brutia* seeds. The 100 % leaf extract of *E. camaldulensis* and 50 % of *P. brutia* increased the mitotic activity and cell division in the meristematic root tip cells of *P. brutia* seeds than control. The leaf extract of *E. camaldulensis* at lowest concentration (25%) were most cytotoxic due to changes in the aberrant chromosome structure of *P. brutia* cells. While, the *P. brutia* leaf extracts (25 % and 100 % concentrations) were highly allelopathic and reduced the mitotic activity, affecting the seedling development in *P. brutia*. Consequently, the results suggested that treatments EC-100 (18 %), PB-50 (14 %) and PP-25 (13.6 %) were very successful to alleviate genotoxic effects of leaf extracts compared to the mitotic index. These groups have a cytoprotective role to protect the stability of chromosome structure during the cell division for *P. brutia* regeneration.

Key Words: Afforestation, allelopathic effects, cell division, *Eucalyptus camaldulensis* Dehnh., fire, genotoxicity, leaf extract, mitosis, *Pinus brutia* Ten., *Pinus pinea* L., seed germination, seedling growth.

INTRODUCTION

Pinus brutia Ten. is one of the major vegetation types in low-altitude forests in the Mediterranean Basin (45). It covers > 4.0 m.ha in the eastern Mediterranean region and widely distributed in Turkey (15). Fire is an effective driver for vegetation dynamics in *P. brutia* forests and other Mediterranean ecosystems (26). Such forests re-generate well after fire, by seeds released from the serotinous cones, which are adapted to forest fires. After the fires the *P. brutia* seeds and seedlings to achieve quick regeneration (49). Brutia pines are preferred for afforestation because of their (i). rapid regeneration capability in post-fire areas, (ii). use for wood production and (iii). soil protection (20). In some cases, however, different fire resistant tree species could be used in afforestation, to change the land cover and create more fire-resistant and resilient landscapes, thus increasing the heterogeneity and landscape barriers to prevent the spread of fire (15). Non-native species (*Pinus* and *Eucalyptus*) plantations have been used in the Mediterranean Basin (2,42). The

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replacement of native species with exotic species leads to wide range of ecological impacts on native flora and fauna (32). The plantations have negative or positive effects on organisms (7,11,14). In fact, these effects have raised concerns for ecosystems and restoration of native vegetation in plantations to protect the resources and the local fauna (14). In afforestation of natural forests, exotic species used in plantations may affect the success of re-colonization of native species by (i). altering the physical and chemical properties of soil (7,30) and (ii). affecting the seed germination and seedling development (53). Most of these studies investigated the negative allelopathic interactions of exotic species plantation on native species (17,19,50,52). Some conifer and particularly *Eucalyptus* species release allelochemicals which have negative allelopathic effects on the development of native species. However, the findings of this study, showed that some exotic species (e.g. *Eucalyptus camaldulensis* Dehnh.) used in afforestation of post-fire *P. brutia* forests.can have facilitating effects on the native species.

Mitotic inhibition is one of the important mechanisms in the allelopathic interactions (6,37,38). Cytogenetic monitoring is the most effective method for understanding species-specific effects of the cell cycle at the DNA level. It allows the determination of cytogenetic changes via mutagenesis. The mitotic index is a measure of the mitotic cycle, which can be measured as a degradation of nuclear activation or a deterioration of cellular homeostasis (10,25). Reduction in mitotic activity might be due to the prevention of a cell from entering into M phase (Mitotic phase), due to inhibition of DNA synthesis or a blocking of the G2 phase of the cell cycle (44). Many studies have tested various concentrations of leaf extracts to find an optimum dose for stimulating mitosis and plant growth (12,35). Conversely, there have been several studies to determine leaf extract inhibitors and their clastogenic and mutagenetic effects on the cell cycle and chromosomal structure (33,36).

This study aimed to investigate the allelopathic effects of planting of exotic species (*P. pinea* and *E. camaldulensis*), on cell division and chromosome structure during the seed germination and seedling development of *P. brutia.*

MATERIALS AND METHODS

Leaf Extraction

Leaf samples of *E. camaldulensis, P. brutia* and *P. pinea* were collected from the reforestation and afforestation sites (36° 51' 05" N, 28° 13' 23" E) growing in *P. brutia* forests destroyed by fire in 1996. The sites are located near the northeastern border of Marmaris National Park (Marmaris, Muğla, SW Turkey) and extend from 0 to 250 m. This area has typical Mediterranean climate with hot and dry summers. The total precipitation is 1211.7 mm $year^{-1}$ (1975-2006), with 5-months (May-September) dry period. Mean monthly temperatures: 10.6 °C in January to 28.3 °C in July.

The sampling of leaves from 5-trees of each species was done in June 2016. The air-dried leaves were powdered prior to extraction. Powdered leaves (35 g) were extracted with 350 ml ethanol (ETOH 96%) by stirring in water-bath at 40°C for 24 h. Before the ethanolic extract was filtered, the residue was re-extracted thrice. Filtered extracts were evaporated to dryness using a rotary evaporator and then re-dissolved in 100 ml distilled water.

There were 10-Explerimental treatments: EC-100, EC-50, EC-25, PB-100, PB-50, PB-25, PP-100, PP-50, PP-25. The full forms of these Abbreviations are: PP: *Pinus pinea*, EC: *Eucalyptus camaldulensis*, PP: *Pinus brutia*.

Cytogenetic Analysis

Fifty uniform-sized *P. brutia* seeds were placed on filter paper sheet in each 9 cm dia Petri dish and covered with two sheets of filter paper moistened with 5 ml of distilled water or leaf extracts (5 ml of 25, 50 and 100 % solutions; control: 0 %) as per treatments. The petri dishes were kept in growth chamber (20° C, 12 h light/12 h dark) for seeds germination. When the root tips were 1-1.5 cm long, they were cut off, pre-treated with ice water for 24 h, then fixed for 24 h in Carnoy solution (3:1, 99 % ethanol to glacial acetic acid) and stored in 70 % ethanol at 4°C until required. The root tips were hydrolysed with 1 N HCl for 10 min, soaked in Feulgen stain for 1 h and then chopped in drop of 45 % acetic acid on slides (54). Three root tips were examined for each leaf extract concentration and control group. The mitotic index, phase indices (*I*) of dividing cells and chromosome aberrations were scored by analysing at 3,000 cells per treatment (1,000 per slide). The mitotic index was calculated as under:

Mitotic index (MI) = Number of cells in mitotis / Total number of cells

To compare the cell division in more detail, the indices of the separate phases were calculated: Prophase (I_P), Metaphase (I_M), Anaphase (I_A) and Telophase (I_T). According to Ivanova *et al*. (2002), phase indices were examined as under:

I_{phase} = Number of cell of the respective phase/Total number of divided cells.

The cytotoxicity and genotoxicity of chromosomal aberrations were determined by calculating the number of aberrant cells by the total number of divided cells. The abnormal chromosomes were observed at 100x magnification with a Carl-Zeiss Axioscope trinocular light microscope and were photographed using Canon EOS650D camera.

Statistical Analysis

Data from the leaf-extract control (0 %) and treated (25, 50, 100 %) samples were compared using ANOVA with MiniTab v. 17 and SPSS v. 22 software. The differences between each group were evaluated using non-parametric Kruskal-Wallis analysis and Duncan's multiple range test, at a level of significance of $p \leq 0.05$ (Duncan 1955).

RESULTS AND DISCUSSION

Effects of Leaf Extracts on Mitotic Activity

The effects of various concentrations (0, 25, 50, 100%) of the leaf extracts of *E. camaldulensis* (EC), *P. brutia* (PB) and *P. pinea* (PP) on the mitotic index and mitotic phases in the root tip cells of *P. brutia* are shown in Table 1. The extracts of these species were stimulatory/inhibitory to mitotic index of *P. brutia*.

All concentrations of the *P. pinea* leaf extract (PP-25, PP-50 and PP-100) inhibited the mitotic activity in *P. brutia* seeds during seed germination. Mitotic indices of all these concentrations of this species were lower than control group. At the same time, the lowest prophase index except for the control group was found at PP-25 (Table 1).

The *P. brutia* leaf extracts applied at highest and lowest concentrations (PB-100 and PB-25) to *P. brutia* seeds did not stimulate the mitotic index. On the other hand, 50 % concentration of *P. brutia* leaf extract was found to have higher mitotic index value than the control group (Table 1 and 3).

The highest concentration (100 %) of *E. camaldulensis* leaf extract increased the mitotic activity compared to control group. EC-100 led to 2-folds increase in the prophase index (0.84) on the root tips of *P. brutia* compared to control (0.45), however, the lower concentrations of *E. camaldulensis* (EC-50 and EC-25) had lower mitotic and metaphase index values than control group (Table 1).

Table 1. Mitotic index and phase indices are shown against concentration decrease in threated with leaf extract concentrations (100, 50 and 25 percent) and untreated (control, 0 percent) groups of *P. brutia.*

Treatment	**Mitotic Index (MI)**	**Prophase Indice (Ip)**	**Metaphase Indice (IM)**	**Anaphase Indice (IA)**	**Telophase Indice (IT)**
Control (0)	* 0.47±0.29bc	0.45±0.16a	0.18±0.08c	0.03±0.02a	0.33±0.18b
EC-100	0.50±0.16c	0.84±0.03c	0.06±0.03a	0.01±0.01a	0.04±0.03a
EC-50	0.33±0.15abc	0.57±0.08ab	0.11±0.08abc	0.01±0.01a	0.16±0.12a
EC-25	0.28±0.05abc	0.57±0.20ab	0.04±0.02a	0.02±0.01a	0.04±0.05a
PB-100	0.16±0.03a	0.61±0.14ab	0.12±0.08abc	0.03±0.04a	0.16±0.10a
PB-50	0.49±0.23c	0.79±0.08bc	0.07±0.01ab	0.01±0.02a	0.04±0.03a
PB-25	0.16±0.01a	0.56±0.13ab	0.17±0.06bc	0.02±0.02a	0.07±0.07a
PP-100	0.33±0.07abc	0.76±0.15bc	0.10±0.05abc	0.01±0.02a	0.02±0.02a
PP-50	0.24±0.03abc	0.66±0.08abc	0.09±0.05abc	0.02±0.01a	0.01±0.02a
PP-25	0.22±0.02ab	0.50±0.01a	0.12±0.02abc	0.04±0.03a	0.02±0.01a

* Values with insignificant difference ($P \leq 0.05$) for each column are indicated with same letters (means ± SD).
EC: *Eucalyptus camaldulensis*, PB: *Pinus brutia*, PP: *Pinus pinea*

The decrease in mitotic activity in leaf extract at high concentrations of *P. brutia* and *P. pinea* can be correlated to the potential inhibitory effects (5,41) or blocking the mitotic phases. The > 50 % decrease in the mitotic index over the control is the upper cytotoxic limit of test species and may be classed as the cytotoxic limit for *P. brutia*; however, the lowest concentration (PB-25) also showed same cytotoxic limit value. Some leaf extracts decreases the mitotic activity (13), blocks the synthesis of DNA (29,40), or inhibits the mitosis (3). Direct interaction of leaf extract at high concentrations can damage the genetic material, giving rise to critical mutational effects.

Regarding mitotic phases, prophase indices in all applied extracts were higher than control group. EC-100 and PB-50 followed the trend in terms of prophase indices similar to mitotic indices at highest concentrations among all the groups. PP-100 stimulated the mitotic cycle with high prophase index value but did not stimulate the mitotic activity (Table 1). In almost all applied extract concentrations, metaphase, anaphase and telophase

indices values were lower than control. An exception to this was found at PP-25. A slight increase (0.04) for this group was recorded over control (0.03) (Table 1).

Genotoxic Effects of Leaf Extracts on Chromosome Structure

The potential genotoxic effects of leaf extracts on *P. brutia* chromosomes are shown (Table 2) with different types of aberration indices for each mitotic phase. The cytogenetic and genotoxic effects of leaf extracts of *E. camaldulensis*, *P. brutia* and *P. pinea* were observed by determining mitotic activity and chromosome aberrations (disorderly prophase, metaphase plate, stickiness, lagging and uncoiling chromosomes, alignment anaphase, and anaphase and telophase bridges). The control group (0% leaf extract, distilled water) showed the normal mitotic phases (Figure 1a-d). Genotoxicity indices of all studied groups except PB-100 and PP-25 were significantly higher than control (Table 3). The mitotic activity was inhibited at all concentrations of extracts except for EC-100 and PB-50, which, showed many cytotoxic aberrations (Table 1). The genotoxicity index, or chromosome aberration index, decreased with increase in leaf extract concentration. The highest genotoxicity index (EC-25) occurred at the lowest leaf extract concentrations (25%) of these three test species except *P. pinea*. It is apparent that *E. camaldulensis*, *P. brutia* and *P. pinea* leaf extracts at high concentrations had effects on mitotic phases at leaf extract. This is why the genotoxicity index decreased in all high concentrations of leaf extracts (Table 2).

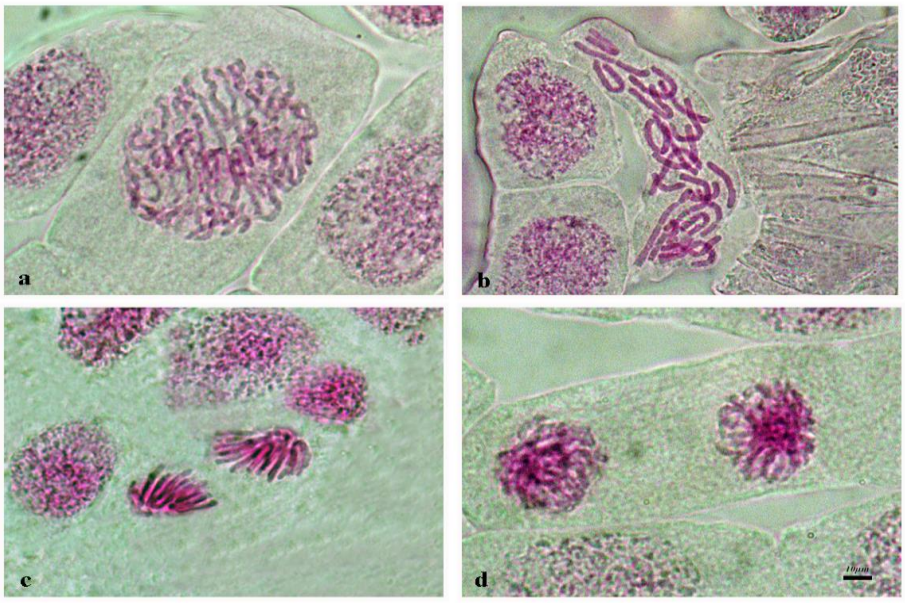

Figure 1. Normal mitotic chromosome structure in the meristematic root tip cells of *P. brutia* germinated in distilled water (control, 0% leaf extract concentration of *E. camaldulensis*, *P. pinea* and *P. brutia*) group. Prophase (a), metaphase 2n=24 (b), anaphase (c), telophase (d). Scale Bar: 10 µm

Table 2. Genotoxicity index (I_G) and aberration indices of chromosomes are shown against concentration decrease in threated leaf extract concentrations (100, 50 and 25 percent) and untreated (control, 0 percent) groups of *P. brutia*.

Treatment	Genotoxicity Index (IG)	Disorderly Prophase	Stickiness	Uncoiling Chromosome	Metaphase Plate	Alignment Anaphase	Fault polarization	Anaphase / Telophase Bridge	Lagging Chromosome
Control (0)	* 0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	**0.00±0.00a**
EC-100	0.09±0.05abcd	0.02±0.01ab	0.01±0.01a	0.01±0.01a	0.00±0.00a	0.02±0.03a	0.03±0.04a	0.01±0.01ab	**0.01±0.01a**
EC-50	0.16±0.07bcd	0.01±0.02ab	0.01±0.02a	0.02±0.03b	0.01±0.02a	0.01±0a	0.06±0.03a	0.00±0.00a	**0.00±0.00a**
EC-25	0.31±0.08e	0.09±0.03d	0.06±0.05b	0.04±0.01b	0.02±0.01a	0.02±0.02a	0.09±0.04a	0.01±0.01ab	**0.01±0.01a**
PB-100	0.06±0.04ab	0.00±0.00a	0.01±0.01a	0.01±0.01a	0.00±0.00a	0.00±0.00a	0.06±0.06a	0.00±0.00a	**0.01±0.01a**
PB-50	0.07±0.02abc	0.01±0.01a	0.01±0.01a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.03±0.02a	0.00±0.00a	**0.01±0.01a**
PB-25	0.23±0.02de	0.03±0.03abc	0.01±0.01a	0.00±0.00a	0.01±0.02a	0.02±0.01a	0.08±0.06a	0.02±0.02b	**0.02±0.01a**
PP-100	0.11±0.05abcd	0.01±0ab	0.01±0.01a	0.01±0.01a	0.00±0.00a	0.00±0.00a	0.07±0.06a	0.01±0.01ab	**0.01±0.02a**
PP-50	0.21±0.03cde	0.06±0.04bcd	0.03±0.04b	0.01±0.01a	0.01±0.01a	0.02±0.02a	0.07±0.05a	0.00±0.00a	**0.02±0.02a**
PP-25	0.03±0.03de	0.08±0.05cd	0.01±0.01a	0.01±0.01a	0.00±0.00a	0.00±0.00a	0.04±0.03a	0.01±0.01ab	**0.02±0.02a**

Values with insignificant difference ($P < 0.05$) for each column are indicated with same letters (means ± SD).

Table 3. The multiple least significant comparison results among treatment groups of mitotic activity and genotoxicity.

		Mitotic activity (p value)									
		EC-100	EC-50	EC-25	PB- 100	PB-50	PB-25	PP-100	PP-50	PP-25	Control
Genotoxicity (p value)	EC-100	-	0.158	0.063	**0.008**	0.931	**0.007**	0.142	**0.033**	**0.021**	0.805
	EC-50	**0.036**	-	0.623	0.150	0.183	0.142	0.954	0.421	0.316	0.237
	EC-25	**<0.0001**	**<0.0001**	-	0.330	0.075	0.316	0.664	0.750	0.603	0.101
	PB-100	0.320	**0.004**	**<0.0001**	-	**0.009**	0.977	0.166	0.507	0.644	**0.013**
	PB-50	0.616	**0.012**	**<0.0001**	0.616	-	**0.009**	0.166	**0.04**	**0.026**	0.873
	PB-25	**<0.0001**	**0.045**	**0.024**	**<0.0001**	**<0.0001**	-	0.158	0.489	0.623	**0.012**
	PP-100	0.548	0.118	**0.004**	0.118	0.275	**0.001**	-	0.454	0.344	0.216
	PP-50	**0.002**	0.200	**<0.0001**	**<0.0001**	**0.001**	0.424	**0.008**	-	0.839	0.055
	PP-25	0.098	**0.001**	**<0.0001**	0.484	0.235	**<0.0001**	**0.029**	**<0.0001**	-	**0.036**
	Control	**0.012**	**<0.0001**	**<0.0001**	0.098	**0.036**	**<0.0001**	**0.003**	**<0.0001**	0.320	-

Values with bold type indicate that difference between two groups is statistically significant.

For instance, the chromosome aberration values were 0.31 at EC-25, 0.23 at PB-25 and 0.21 at PP-50, but rates were decreased to 0.09, 0.06 and 0.11 respectively, at 100% concentrations. In general, the highest concentrations had the lowest genotoxicity index values in all leaf extracts except *P. pinea*. In tandem with increasing leaf extract concentrations, chromosomal aberrations also increased (Table 2). Many studies have reported that chromosomal aberration is highly dependent on increasing leaf extract concentration (1,28,43,46). Depending on the species, an individual component of leaf extract changes the level of clastogenecity in the chromosome structure (27).

Observations were made for all phases of *P. brutia* seeds, germinated with different concentrations of each leaf extract, under the microscope to record features such as disorderly prophase (Figure 2a-d), sticky chromosomes in metaphase (Figure 3a-d), uncoiling chromosomes (Figure 4a, b), metaphase plates (Figure 4c, d), lagging chromosomes (Figure 5a-f), alignment anaphase (Figure 6a, b), fault polarisation (Figure 6c-h). In control no aberrant chromosomes or genotoxic cells were found and all mitotic cells were normal. The highest phase aberration rates, with 0.09 in the meristematic root tip cells of *P. brutia*, were disorderly prophase (Figure 2) and fault polarisation (Figure 6) at EC-25. All leaf extract concentrations, except PB-100, showed significant effects on disorderly prophase; however, no aberrant cells were found in that phase. Sticky and uncoiling chromosomes were the secondary major aberrations, at EC-25, among all treated groups. Metaphase plates, alignment anaphase and lagging chromosomes were counted for all leaf extract concentrations, but they were not significant ($P \leq 0.05$). Bridges in the anaphase or telophase stages were fewer than those of disorderly prophase, stickiness and uncoiling chromosomes.

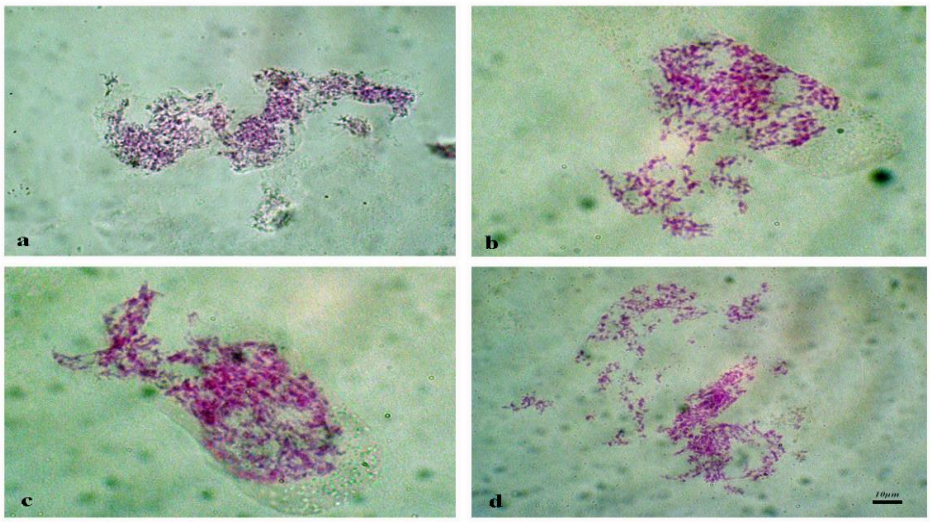

Figure 2. Disorderly prophase aberrations (a-d) in the meristematic root tip cells of *P. brutia.* germinated in 25, 50, 100% leaf extract concentrations of *E. camaldulensis*, *P. pinea* and *P. brutia.* Scale Bar: 10 µm.

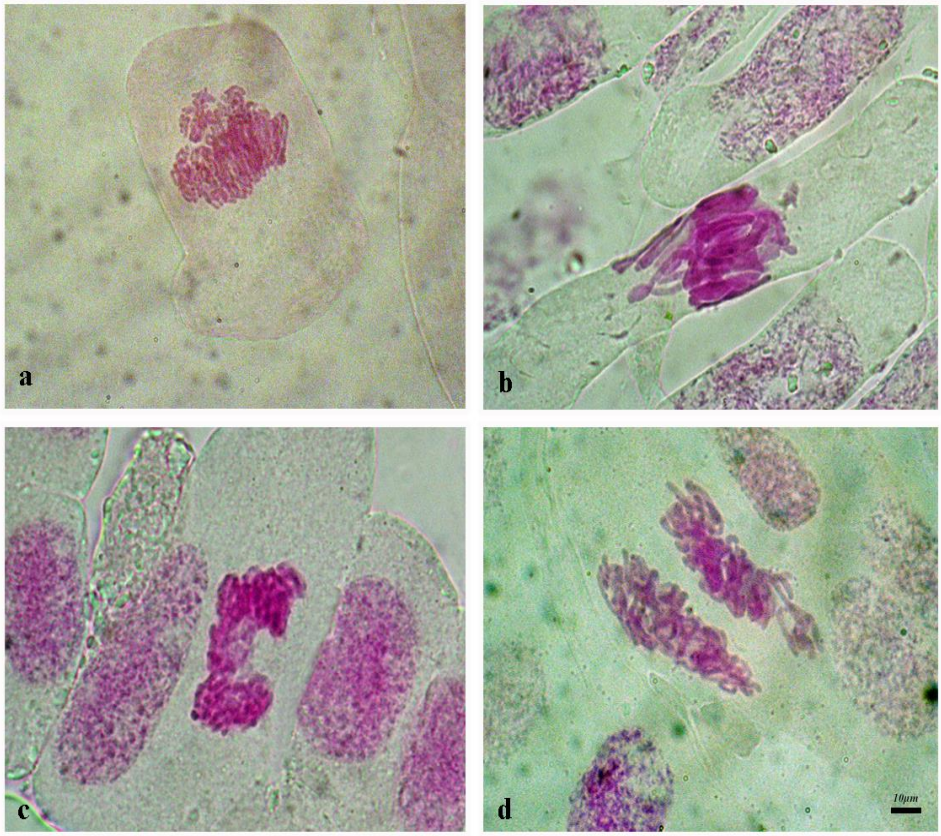

Figure 3. Sticky chromosome aberrations (a-d) in the meristematic root tip cells of *P. brutia* germinated in 25, 50, 100% leaf extract concentrations of *E. camaldulensis*, *P. pinea* and *P. brutia.* Scale Bar: 10 µm

Briefly, the genotoxicity index decreased the tandem in *P. brutia* with an increase of leaf extract concentration, while, lower concentration of *E. camaldulensis* was inhibitory. Chromosomal aberrations decreased at the highest leaf extract concentrations than lower concentration indexes (Table 2). However, the lowest concentration of *P. pinea* leaf extract had significant ($P \leq 0.05$) detrimental effects. Likewise, EC-25 also proved potential genotoxic to *P. brutia.* Chromosomal aberrations were observed across wide range of structures in the chromosomes, resulting in disorderly prophases, sticky chromosomes, metaphase plates, uncoiling and lagging chromosomes, alignment anaphase, fault polarisation and anaphase/telophase bridges (Figures 2-6). It was noted that the index rates of aberrations were statistically important when observations of major disorderly prophase, sticky chromosomes and fault polarisation were made. Gaulden (22) implied that sticky chromosomes were characterised by the loss of function of one or two types of specific non-histone proteins that control chromatid separation and segregation.

Exposure of genetic material to a mutagenic agent is caused by the functional and structural mutation of such proteins (48). The stickiness shows high toxicity that causes inappropriate protein-protein interactions (34,47).

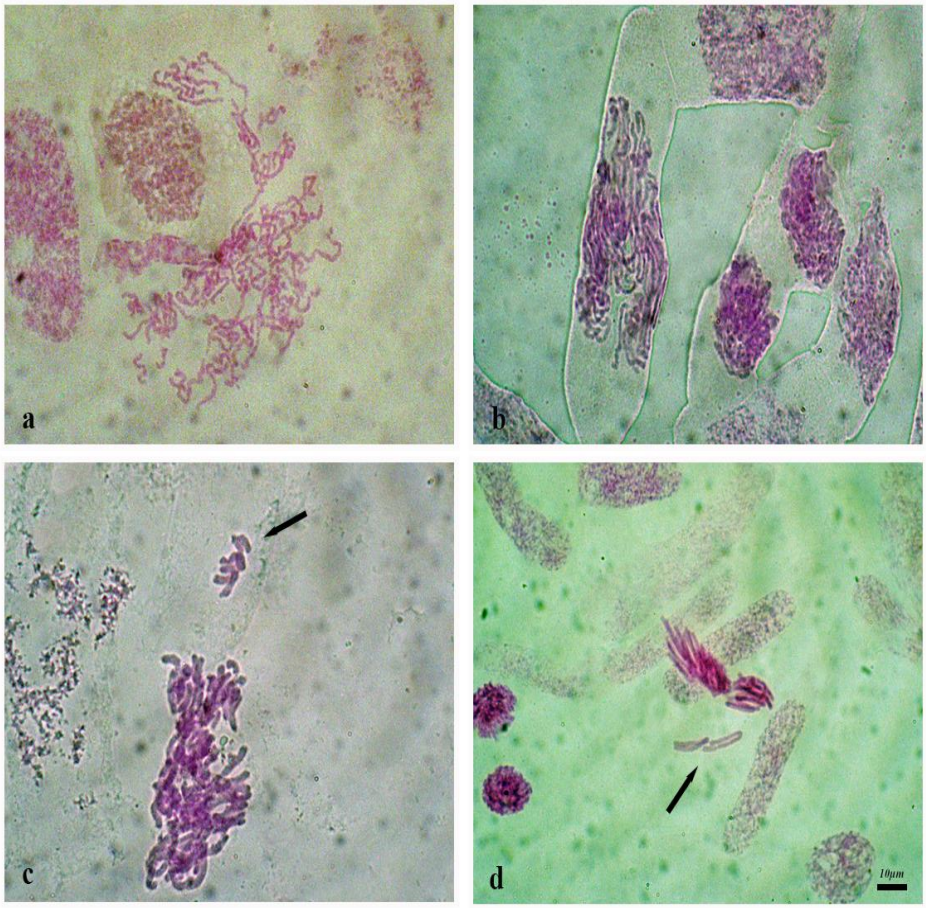

Figure 4. Metaphase aberrations in the meristematic root tip cells of *P. brutia* germinated in 25, 50, 100% leaf extract concentrations of *E. camaldulensis*, *P. pinea* and *P. brutia* (a-b) uncoiling chromosomes. (c-d) laggings and disrupted metaphase plate. (→ represent aberrant chromosome). Scale Bar: 10 µm.

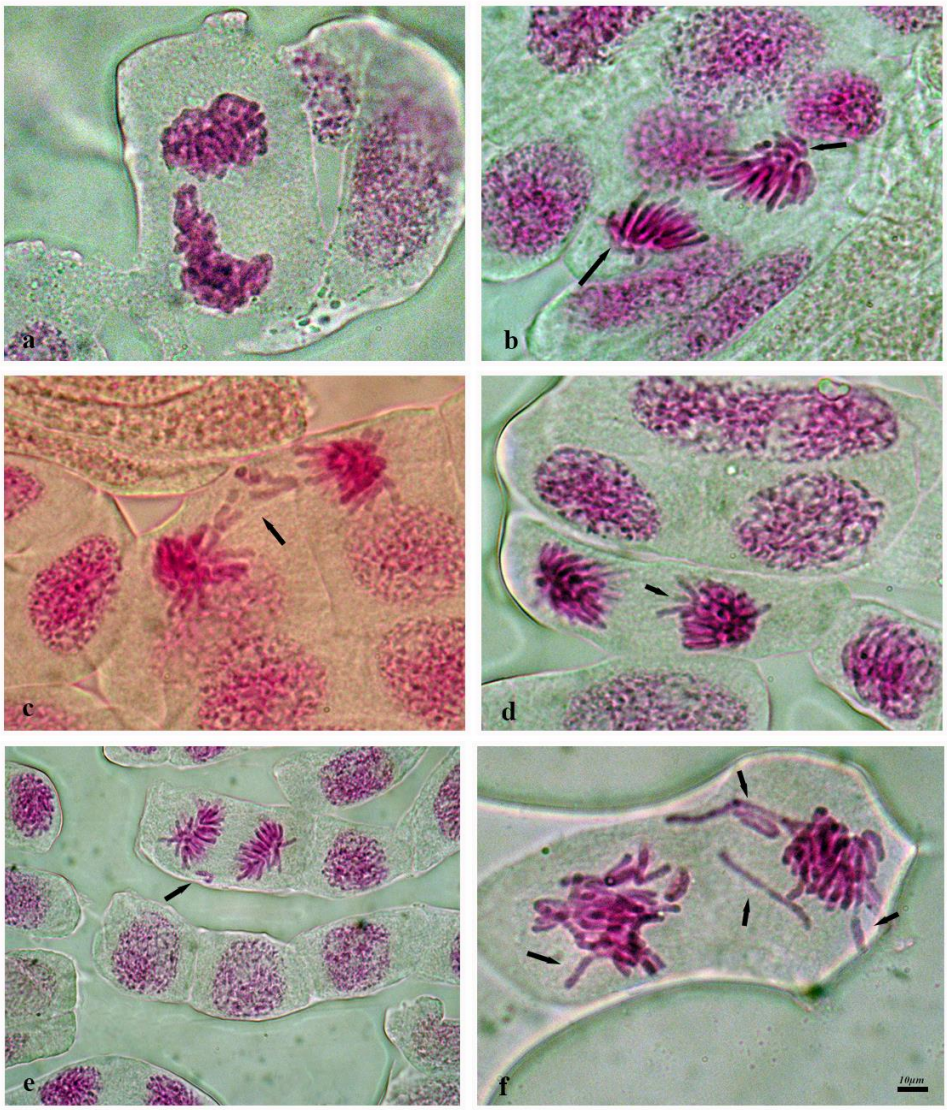

Figure 5. Anaphase aberrations in the meristematic root tip cells of *P. brutia* germinated in 25, 50, 100% leaf extract concentrations of *E. camaldulensis*, *P. pinea* and *P. brutia* a) disorderly anaphase (b-f) laggings chromosomes in anaphase and telophase, respectively. (→ represent aberrant chromosome). Scale Bar: 10 µm

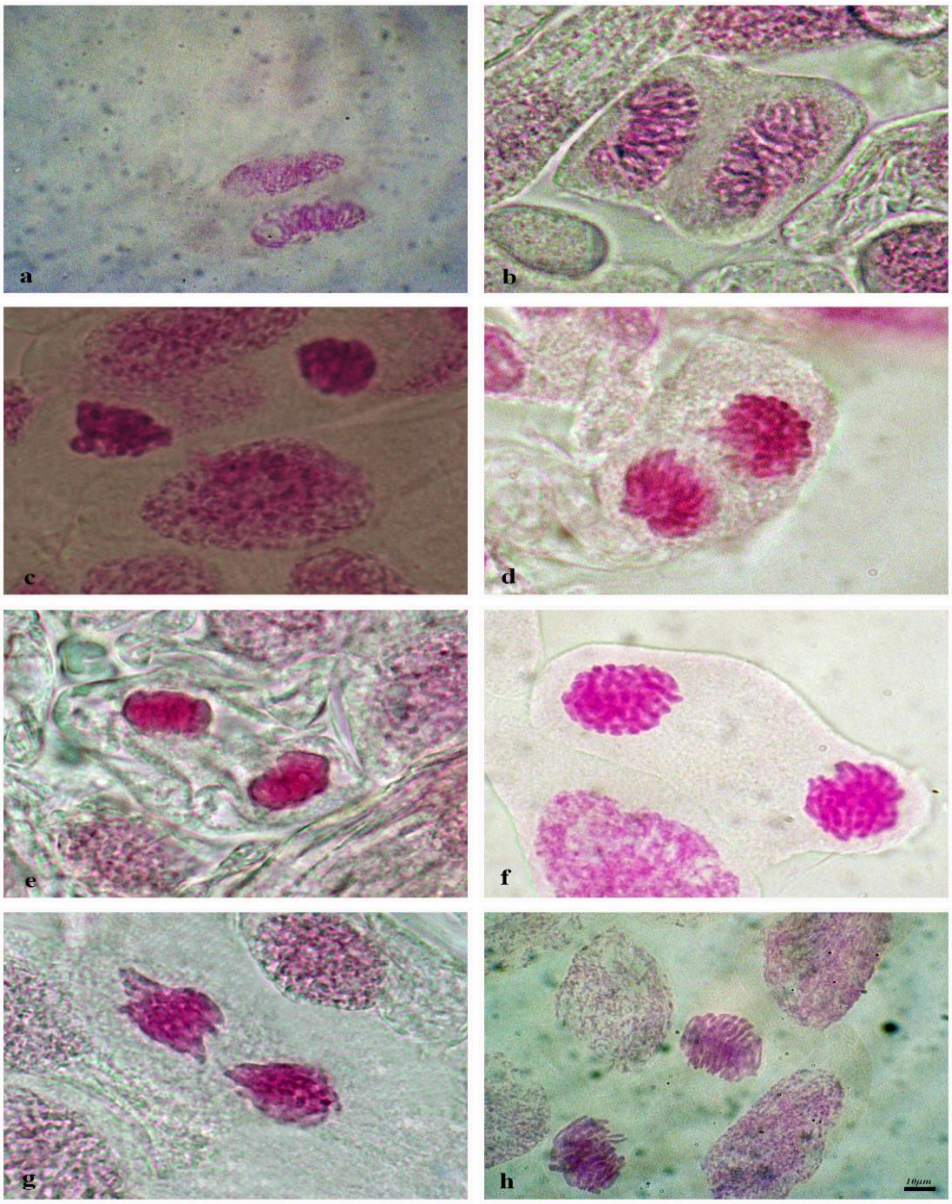

Figure 6. Anaphase and telophase aberrations in the meristematic root tip cells of *P. brutia* germinated in 25, 50, 100% leaf extract concentrations of *E. camaldulensis*, *P. pinea* and *P. brutia* (a, b) alignment anaphase (c-h) fault polarization in anaphase and telophase, respectively. Scale Bar: 10 µm

The highest rate (0.06) of sticky chromosomes was shown by EC-25. Morsi and Abdelmigid (31) also reported that the second major aberration of *E. camaldulensis* leaf extract was stickiness. In contrast to the results presented herein, these authors showed that chromosome aberration rates and mitotic inhibition at higher concentrations were harmful in barley seeds. Certain previous studies have implied that the allelochemicals in *Eucalyptus* leaf extract inhibits the cell division and the cell elongation with changing values depending on the native plant species in the forest (52). The third most significant aberration was fault polarisation, which, along with lagging chromosomes and alignment anaphase, is a clastogenic effect that causes chromatid breaks and can separate the centromere. Thus, the aberrant chromosomes were near to each other (Figs 5,6). Many studies on the clastogenic effects on chromosomes by leaf extracts have reported the polar deviation, stickiness, fragments, bridges in anaphase and/or telophase, lagging and vagrant chromosomes (4,16,28,51).

Therefore, this study suggested that depending on the *E. camaldulensis* leaf extracts stimulated the cell division and mitotic activity of *P. brutia* seeds. These also detoxifies the genetic material and can ameliorate the detrimental effects at high concentrations. The results revealed that the *E. camaldulensis* leaf extract may regenerate chromatin fibrils but inhibit the harmful impacts on chromosome structure at higher increasing concentrations.

The *E. camaldulensis* at the highest concentration, increased the mitotic activity and had some cytotoxic and genotoxic effects on growth of *P. brutia* seeds, the effects of exotic *Eucalyptus* species on natural vegetation and soil characteristics varied considerably (8,9,39). While in some studies, negative allelopathic effects were detected (6,52), many other studies reported stimulatory effects on habitat quality of sites (23,24). Some *Eucalyptus* species were evaluated as a nurse-tree species to promote natural vegetation and recovery of tropical forests (14). The findings obtained about the mitotic index in this study supported positive remarks about *Eucalyptus* species. At the beginning of growth, the effect of increasing cell division may be one of the facilitating mechanisms of *Eucalyptus* species on natural vegetation. However, the negative impact on genotoxicity should not be ignored when making a definitive evaluation. Unlike *E. camaldulensis*, the other exotic species used in afforestation, *P. pinea*, was found highly allelopathic to seedling development of *P. brutia*. The inhibitory effects of *P. pinea* on seed germination and seedling recruitment of Mediterranean shrub species had been reported (50). The findings showed that *P. brutia*, the predominant native tree species of the area, had autotoxic effects on the growth of its own seeds and seedlings. Autotoxicity was evaluated at a functional process that could influence the natural regeneration of some *Pinus* species (21). Therefore, in the silvicultural applications such as clear cutting for the natural regeneration of *P. brutia*, allelopathic chemicals accumulated in the soil might play important role.

CONCLUSIONS

Stimulatory and inhibitory effects on mitotic activity of *P. brutia* in different concentrations of all relevant species were determined. However, the findings suggest that the stimulating effect of *P. brutia* and *E. camaldulensis* is more prominent in this regard. Positive effects of *E. camaldulensis* on *P. brutia* seed germination and seedling recruitment may facilitate the introduction and re-colonization of *P. brutia* into post-fire

afforested areas. However, *P. pinea* species should be used with caution due to its genotoxic and cytotoxic effects at high concentrations along with the inhibitory effects on mitotic activity. More extensive studies are needed to determine the effects of exotic species on the growth of native species.

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