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# Naphthalene induced "Cross-Talk" between pollen mother cells in *Vicia faba* L. plants

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## Abstract

A cytomixis is a common event that affects the male gametogenesis process and is influenced by several factors. Despite the fact that cytomixis has been documented earlier, there are still some unanswered questions about the origins, significance, and genetic regulation of cytomixis. The transmigration of genetic materials between pollen mother cells (PMCs), via cytoplasmic channel and direct fusion, was detected during investigated meiosis division in naphthalene (NAP) induced Vicia faba L. plants with 1,3,5 and 7 g/L. Furthermore, along with cytomixis, a variety of chromosomal abnormalities were seen, such as chromosomal stickiness, laggards, chromosomal bridges, uneven chromosomal separation, micronuclei formation, and chromosomal loss accompanied with meiotic index reduction. Cytomixis is assumed to be the cause of a significant portion of deformed pollen, which led to pollen sterility. Additionally, it was demonstrated that the post-meiotic product was significantly impacted by naphthalene induction. There were irregular sporads like triads, dyads, monads, and polyads as well as ordinary tetrads. The largest chromosomal aberrations were correlated with higher amounts of mutagenic agent application. We also noticed that the number of aborted seed and ovules was significantly affected at higher concentrations of the NAP applications. It is evident that cytomixis and meiotic abnormalities affect the percentage of pollen fertility, highlighting the association between this parameter and the occurrence of cytomixis. Our results demonstrate the NAP toxicity are almost the only factors identified in this study as contributing to cytomixis. Cytomixis may result in evolutionary significances as it can produce pollen grains with diverse genetic content. This could have an impact on evolution and could be beneficial for projects aimed at agricultural progress.

**Keywords:** Cytomixis; Syncytes; Pollen Viability; Naphthalene; Seed abortion; Comet assay

## 1. Introduction

Multiple meiotic abnormalities, including cytomixis, were found for the first time at the diploid plant in the PMCs of *Vicia faba* L [1]. Cytomixis, as defined by Bhat [2] and [3], is the process of chromatin transfer through cytoplasmic connection channels between adjacent cells. It has also been shown that direct cell fusion occurs in some incredibly rare situations. According to several studies [4,5.6], cytomoxis is present in many plant species. Although it has also been observed in the tapetal and ovary cells of several plants, it is predominantly observed in pollen mother cells (PMCs) [7]. The majority of the time, this cytological phenomenon has been observed during the microsporogenesis of genetically unequally cells, forming haploids, aneuploids, polyploids, apomicts, hybrids, and among other multiple genomic plants [8]. Cytomixis can have deleterious impact on cell division process, such as chromosome stickiness, syncytia, pollen sterility or decreased male fertility [9], aberrant microspores (dyads, triads, pentads, hexads, and heptads) and PMCs with abnormal chromosome numbers. [10] states that each of these abnormalities has a negative impact on the capacity to reproduce and may even change the reproductive system. Cytomix phenomenon can be induced via a variety of external triggers, in plants. The primary factors leading to cytomixis include temperature impacts, environmental stresses and pollution[10,11]. Polycyclic aromatic hydrocarbons, or PAHs, are the most important organic contaminants in environmental study. They may have mutagenic and carcinogenic effects in addition to their high potential for toxicity

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and environmental persistence. The aromatic (benzene) rings that bind with the carbon and hydrogen atoms that make up PAHs can result in either linear or branched compounds [12]. Out of theses PAHs, 16s are the focus of extensive research due to their importance in toxicity, industry, and the environment pollution. Naphthalene (NAP) among this category, described as a bicyclic aromatic hydrocarbon that naturally arises from coal tar or crude oil, is one of the members of this particular group[13]. NAP can enter the environment from a variety of sources, including car exhaust, roads, coal, coal tar, wildfires, residential wood burning, agricultural burning, incineration of commercial and industrial trash, and toxic waste sites. NAP can cause a variety of cellular and systemic reactions when it comes into contact with aquatic life [14]. Plants and animals that are contaminated may build up in the food chain or sediment, which could expose humans and other animals inadvertently. To detect environmental mutagens, numerous plant bio-assay have been evolved, owing to the fact that plant chromosomes are easy to observe and respond to mutagen treatment in a manner comparable to that of other eukaryotes [15,16,17]. These are also effective bioindicators of biochemical parameters and the potential for contaminants to cause cancer and mutagenesis. Consequently, utilizing faba plants as the experimental model [18], our study was aimed to comprehend the impact of exposure of faba seeds and buds to different concentrations of the most widely used home fumigant, NAP. Additionally, to demonstrate the relationship between pollen fertility and cytomixis, as well as to explore the consequences of syncytes production through cytomixis transmigration in treated faba plants.

## 2. Material and Methods

### 2.1. The Source of experimental plants

Healthy and dry seeds of Vicia faba L., common name (broad bean) 2n=12, were purchased from the local market. After sterilization, the seeds were treated with the different concentrations of naphthalene (0.0, 1.0, 3.0, 5.0, 7.0, 10 mg/l) for (6, 12, 18, 24 hours) and then allowed to soak in distilled water for a full day. Seven seeds from each treatment were transferred from individual Petri plates to pots during the first week of November., The seeds/seedlings were grown in mixture of soil and vermiculate with sand 1:1 kept at a temperature of 22±2 °C. The experiment was performed in three independent replicates for genetic and cytogenetic analyses.

### 2.2. Cytogenetic analysis

#### *2.2.1. meiotic index (MI) and meiotic stages abnormalities*

For analysis of microsporgenesis process, buds were separated from plants and sequentially arranged based on their position and size on the floral buds. Flowers buds were dissected on microscope slide into 2 drops of 2 % acetocarmine solution using a dissecting microscope (Zeiss). Anthers were gently pressed with tiny forceps and dissecting needle to release the micrspores, coverslip was mounted and gently pressed to squash the samples, sealed and visualised under microscope(Olympus BX41). Number of PMC, tetrads as well as meiotic abnormalities on a slide were recorded

MI wascalculated after the evaluation of more than 400 tetrads using the following formulae [19:

$$\mathbf{Meioticindex(MI)} = \frac{\text{number of normal tetrads}}{\text{total tetrads}} \boldsymbol{x100}$$

Plants that are meiotically stable have a MI of 90%–100%. Any variation from the standard tetrad of four identically sized microspores is considered aberration. This includes anomalies in micronuclei, microspores, triads, polyads, monads, and dyads.

#### *2.2.2. Pollen viability (PV)*

For the pollen viability, small drops of a 2% glycerol-acetocarmine/ Aalexender was placed on the microscopic slides. Few completely opened flowers were placed on slide and carefully extracted a small quantity of pollen by squeezing it with tiny forceps or wooden toothpick. After that, the pollen should be dispersed using gentle circular motions. After mounting the cover slip, slides must stay flat for a few minutes before microscopic analysis.Pollen with a normal oval shape and visible staining were considered viable and fertile, while empty or shrivelled and unstained pollen were considered sterile. PV was determined using the following Formula [20]:

$$\text{PV (\%)} = \frac{\text{number of unstained or empty pollen grains}}{\text{total number of pollen grains}} x100$$

### 2.3. Abortion rate and position of ovule and seed

Following the maturation of faba seeds, 20 mature pods were randomly selected in order to determine the number of normal mature seeds, the number of aborted seeds, and the location of the abortion inside selected silique. The aborted seeds were located in the top, middle, and bottom portions of the ovary. The bottom areas of the ovary was linked to the floral part, while the upper section was situated close to the stigma. Missing seed rate was calculated using the following formula:

$$\text{seed abortion rate} = \frac{\text{number of aborted seeds}}{\text{number of mature seeds} + \text{number of aborted seeds}} x100$$

Note: abortion seeds were undeveloped, fat, unfilled, brownish colour.

### 2.4. Comet assay

Mature faba buds from treated and untreated plants were gathered and chopped, in PBS buffer with a razor or scalpel blade after it has cooled on ice. The chopped buds were evenly arranged on glass slides, and suspended in agarose that was progressively melting. The slides were immersed in alkaline solutions and lysed to extract any proteins and pollen cell membranes after the agarose solidified. After that, during a 20-minute electrophoresis, the DNA from damaged nuclei migrated more swiftly than the DNA from healthy nuclei. After that, Et Br staining was applied, and analysis was done using a fluorescence microscope BX51 (Olympus). The images were examined using the Casp-Lab to calculate the amount of DNA in each comet's tail (Tail DNA%: the percentage of DNA content in the tail relative to the overall DNA content in the comet). Two slides containing fifty comet photos each were used to collect data for each group.

### 2.5. Statistical analysis

The two-way ANOVA test was used for statistical analysis using the CoStat statistical program (CoStat software) to ascertain the significance of variable differences. All analysed data are presented as as mean ± standard error.

## 3. Results

### 3.1. Meiotic analysis

To investigate the effects of NAP on the normal pathway of pollen development, acetocarmine-stained pollen released from single anther of successive bud stages, in both control and treated plants, was examined by light microscopy. Counts were made at several stages of pollen development: microspore, meiosis I, and meiosis II, pollen stages.

#### *3.1.1. Dose and time inhibition on meiotic Index*

Out data showed that treating faba seeds with different concentration of NAP in different groups (0.0,1, 3, 5, 7 and 10g/l) using different incubation times(6, 12, 18 and 24 hrs) resulted in significant decline in MI of bean plants (Table 1). Increasing concentrations of naphthalene significantly inhibited meiotic figures in treated samples in dose- and time-related action.We noticed that as the incubation period increased the potential effect of increases in NAP concentrations was more pronounced and this was significant at $p \leq 0.05$. A clear cell division inhibition was observed particularly at 18 and 24 hrs treatment period for both 7 and 10 g/l of NAP treatments. The meiotic index reached a minimum value of 74.2% after 24 hrs treatment with the 10g/l treatment of NAP compared with 99.5% value in the control.All the concentrations of naphthalene used in the study caused changes in the percentage of phases% compared to the control.

**Table 1** Number and percentages of normal and abnormal sporads formation of microsporogensis analysed after treated with naphthalene

| Treats (g/l) | Duration (hours) | Normal tetrads | abnormal sporads % | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Triads | Dyads | Monads | Polyads | total | MI | Sporade% |
| **0** | 6 | 400 | 2 | 0 | 0 | 0 | 2 | 99.5± 2.01 | **0.5** |
| | 12 | 402 | 3 | 0 | 0 | 0 | 3 | 99.3±1.23 | **0.75** |
| | 18 | 399 | 2 | 0 | 0 | 0 | 2 | 99.5±3.03 | **0.50** |
| | 24 | 384 | 1 | 1 | 0 | 0 | 2 | 99.5±2.23 | **0.5** |
| **1** | 6 | 407 | 15 | 0 | 0 | 5 | 20 | 95.3±0.93 | **4.9** |
| | 12 | 409 | 22 | 0 | 0 | 4 | 26 | 94.0±6.21 | **6.4** |
| | 18 | 412 | 20 | 1 | 0 | 7 | 28 | 93.6±5.02* | **6.8** |
| | 24 | 365 | 25 | 3 | 0 | 5 | 33 | 91.7±3.78** | **9.0** |
| **3** | 6 | 329 | 20 | 3 | 0 | 3 | 26 | 92.7±2.73* | **7.9** |
| | 12 | 398 | 28 | 6 | 0 | 6 | 40 | 90.9±5.11** | **10.1** |
| | 18 | 397 | 22 | 6 | 0 | 9 | 37 | 91.5±7.04** | **9.3** |
| | 24 | 402 | 32 | 4 | 0 | 9 | 45 | 89.9±6.35** | **11.2** |
| **5** | 6 | 398 | 28 | 5 | 0 | 2 | 35 | 91.9±3.32** | **8.8** |
| | 12 | 415 | 25 | 4 | 2 | 5 | 36 | 92.0±8.21** | **8.7** |
| | 18 | 401 | 33 | 9 | 2 | 7 | 51 | 88.7±4.49** | **12.7** |
| | 24 | 390 | 25 | 6 | 5 | 7 | 43 | 90.1±4.87** | **11.0** |
| **7** | 6 | 389 | 30 | 8 | 1 | 4 | 43 | 90.0±8.20** | **11.1** |
| | 12 | 398 | 27 | 10 | 3 | 6 | 46 | 89.6±6.14** | **11.6** |
| | 18 | 415 | 33 | 4 | 8 | 6 | 51 | 89.1±3.98** | **12.3** |
| | 24 | 388 | 40 | 8 | 4 | 6 | 58 | 87.0±11.20** | **14.9** |
| **10** | 6 | 398 | 38 | 14 | 7 | 5 | 64 | 86.1±9.03** | **16.1** |
| | 12 | 407 | 38 | 14 | 9 | 6 | 67 | 85.9±11.01** | **16.5** |
| | 18 | 389 | 44 | 12 | 5 | 10 | 71 | 84.6±7.63** | **18.3** |
| | 24 | 390 | 40 | 20 | 10 | 6 | 76 | 83.7±5.74** | **19.5** |
| **total % of categories** | | **9482** | **593** | **138** | **56** | **118** | **905** | | |

Data are mean of three replicates ± SE. MI= meiotic index *p < 0.05 compared to control.

*3.1.2. Abnormal karyokinesis during microsporogenesis*

Anomalies related to meiotic aberrations, such as monads (figure 1B), dyads (figure 1C), and triads(figure1D), were also seen in a some of NAP-treated plants' post-meiotic products. The percentage of normal tetrads(Figure1A) was highest in almost 99% of control plants (Table 1). In contrast, triads were observed in all treated plants and showed a vermiculateconcentration- and time-dependent rise, with the maximum values of 44% at 10% (18 h). Only plants treated with high concentrations of NAP (5, 7, and 10%) exhibited monads. Furthermore, every treated counted sporad exhibited polyads, with plants treated with 10g/l at 18 h displaying the highest proportion of polyads (Table 1).

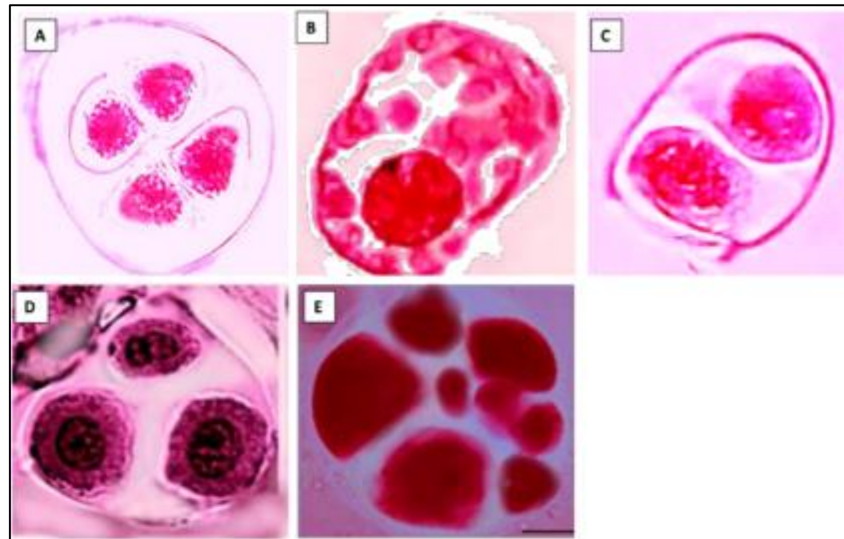


**Figure 1** Some meiotic division deformities. A) normal tetrads B) large polarized monads at mature pollen stage C) Dyads D) unequal segregated triads E) Polyads(pentads

*3.1.3. PMCs showed cyto-morphological irregularities*

In PMCs, the mutagenic treatments resulted in a significant quantity of meiotic aberrations; however, in the control plants, everything appeared normal, having 8 bivalents at metaphase I and normal separation at anaphase and telophase I and II (figure 2). But in plants exposed to NAP, PMCs had a range of chromosomal abnormalities during different meiotic phases. The concentration and duration of the treatment's were discovered to positively correlate with the mutagen-induced meiotic abnormalities, which were found to be dose-dependent. While the spectrum of aberrations was roughly the same for all treatments, the incidence of specific abnormalities varied significantly.

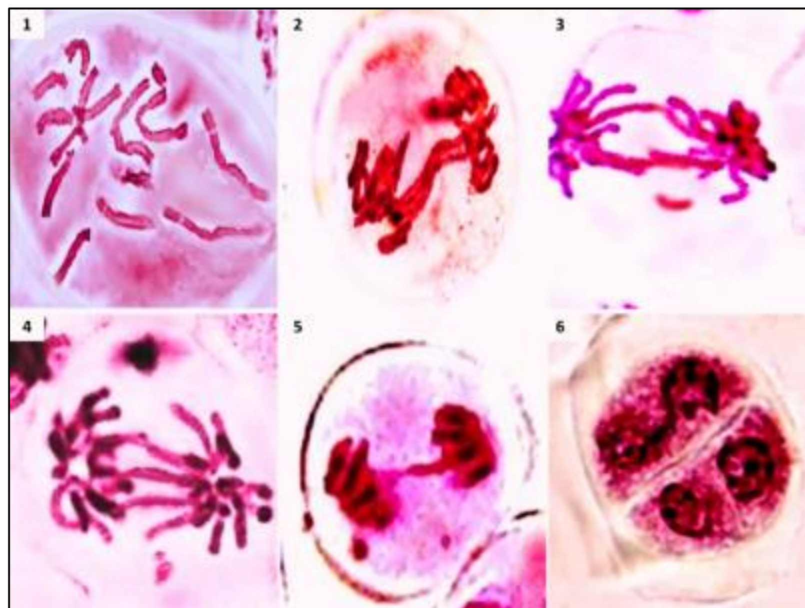


**Figure 2** Example of PMC with meiotic aberrations induced by induced by naphthalene. 1) bivalent Chromosomes of V. faba at control stage. 2) sticky anaphase I with bridges. 3-4) Anphase II with double and triple bridges and chromosomal fragments 5) early telophases I bridges and micronuclei. 6) late telophase II showing abnormal cytokinesis

Interestingly, the chromosome stickiness was seen in every progeny of the mutagenic parent. At 18 hours, sticky PMCs were also observed in control plants (0.24). 10% of NAP (5, 6.7, 7 and 7.44% at 6, 12, 18 and 24 hours, respectively) had the largest percentage of PMCs with sticky chromosomes, followed by 7% (5, 3.9, 0, and 5.54% at 6, 12, 18 and 24

hours, respectively), and much lower in the control group (0.24%).Laggards were also common at various meiotic phases. At 18 and 24 hours, the percentage of laggards was 0.45% and 1.55%, respectively, making up the lowest percentage of 1%. On the other hand, incubation times of 12 and 24 showed that the maximum frequency of lagging chromosomes was 3.2% and 4.2%, respectively, in the 10% naphthalene treatment. then 2,89% at 24 hours of 7% NAP treatment(table 2).

**Table 2** Types and percentages of chromosomal abnormalities induced by different concentrations of naphthalene at different exposure times and duration of treatments of faba plants

| Treat (g/l) | Chromosomal abnormalities % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Duration (hours) | total | stickiness | laggards | Bridges | bivalent | multivalent | total |
| 0 | 6 | 218 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 210 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 18 | 199 | 0.24 | 0 | 0 | 0 | 0 | 0.24 |
| | 24 | 203 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 6 | 211 | 1.4 | 0 | 0 | 0 | 1.8 | 3.2 |
| | 12 | 215 | 1.08 | 0 | 0.37 | 0 | 1.56 | 3.01 |
| | 18 | 210 | 0 | 0.45 | 2.4 | 1.2 | 0 | 4.05 |
| | 24 | 203 | 1.5 | 1.55 | 0 | 2.36 | 0 | 5.41 |
| 3 | 6 | 198 | 2.3 | 2.18 | 2.1 | 0.76 | 2.34 | 9.68 |
| | 12 | 207 | 2.07 | 3 | 0 | 1.21 | 0.78 | 7.06 |
| | 18 | 222 | 2 | 2 | 0 | 1.59 | 0 | 5.59 |
| | 24 | 215 | 3.6 | 1.69 | 1.9 | 0 | 3 | 10.19 |
| 5 | 6 | 211 | 3.3 | 0 | 0.85 | 0 | 2.6 | 6.75 |
| | 12 | 216 | 4 | 1.9 | 2 | 2 | 0 | 9.9 |
| | 18 | 223 | 2.9 | 2 | 0 | 1.86 | 2.78 | 9.54 |
| | 24 | 214 | 0 | 2 | 0 | 1.16 | 2.38 | 5.54 |
| 7 | 6 | 226 | 5 | 0 | 2.3 | 0.36 | 1.98 | 9.64 |
| | 12 | 218 | 3.9 | 0 | 1.18 | 0 | 0 | 5.08 |
| | 18 | 224 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 234 | 5.54 | 2.89 | 1.6 | 0 | 1.4 | 11.43 |
| 10 | 6 | 197 | 5 | 0 | 3.09 | 3.26 | 1.82 | 13.17 |
| | 12 | 204 | 6.7 | 3 | 2.3 | 3.56 | 0 | 15.56 |
| | 18 | 214 | 7 | 0 | 0 | 0 | 2 | 9 |
| | 24 | 189 | 7.44 | 4.2 | 6 | 0 | 2.3 | 19.94 |
| totals | | 5081 | 64.97±0.49 | 26.86±0.27 | 26.09±0.30 | 19.32±0.22 | 26.74±0.231 | 163.98±6.369 |

### 3.2. PMCs exhibiting nucleus migration phenomena

#### *3.2.1. Two types of Cytomixis. Cytoplasmic channels and direct fusion.*

The experimental results presented in table3 and figure3, revealed that most of plants treated with lower concentration of NAP(1,3, 5 and 7 mg/l) were showing cytomictic linkage between two or more PMCs as well as during different phases of meiosis. Nevertheless, in the plants under control, cytomixis was completely absent. Two different kinds of

connections direct fusion(DF- figure 3A) and cytoplasmic channels (CC- figure 3B) between PMCs were noted. CC was seen frequently(361.36) compared to DF (234.21) Fascinatingly, only two of the seven plants treated with 10 mg/l showed signs of CC, with average values of 15.5 and 10.3 after 18 and 24 hours, respectively (Figure 3. A-L). PMCs often underwent cytomixis via cytoplasmic channels (Figure 3, E, J and L). PMCs were also seen to fuse directly at different phases of cell division (Figs. 2, 3, and 10). During the first phase of meiotic division, more cells displayed cytomixis using this DF (Table 2). Chromatin material travelled from one cell to another either partially or completely (Figure 3- C and E), resulting into increased or decreased chromosome number in PMCs. Multiple cytoplasmic threads connecting two or more PMCs were also frequently seen without evidence of chromosomal transfer (Figure 3. B,D, H and I). Interestingly, some PMCs were found to have a CC with one PMC and DF with another(Figure H and J). Although it was also shown between PMCs at different phases of division, cytomixis was often seen between PMCs in the same division stages(Figure 3 C). This is commonly seen at the stages of meiosis I as compared to meiosis II (Table3). Interestingly, this phenomenon widely occurred in early meiosis I including prophase I, metaphase I,anaphase I and telophase I , but in lower frequencies in the later meiotic stages (stages of meiosis II). The frequency of PMCs appeared with chromatin transfer was the highest at prophase I (22.99%), followed by the anaphase I stage (17.6%). In addition, the percentage of PMC was also high during metaphase I (16.38%) and telophases II(16.79%%). Cytomixis involving chromatin migration also occurred in telophase I (14.03%), prophase II, metaphase II and anaphase II (12.3%, 11.09 and 12, 46), respectively. Besides, we did not observe clear evidences for chromatin transferring during meiosis II. The journey of chromatin/ chromosome among meiocytes was directionless and indiscriminate. The chromatin or chromosome material can synchronously transfer from one to another or more contiguous PMCs through the CC (figure 3-I). Additionally, we observed that chromatin or chromosomal components from many meiocytes can go to the same cell at the same time (figure 3-G). In certain instances, a single PMC may function as both the donor and the receptor simultaneously (Figure 3 C and F). Furthermore, partial chromatin material transfer from one PMC occurred more frequently in cytomixis than full chromatin transfer. In general, there was clear decrease in the frequency of PMCs showing cytomixis was found with increasing concentrations of NAP(7% and 10%) (Table 3).

### *3.2.2. Effects of NAP on frequency of syncyte cells in PMCs*

Another intriguing observation discovered in meiotic cells treated with NAP was the presence of syncytes. syncytes are produced when two or more PMCs completely congregate and transmit their entire cell content to the recipient PMC (figure 3-M). Consequently, doubled chromatin complement is added to the recipient PMC. In the control group or at the lowest concentrations (1 and 3 mg/l), no syncyte cells were seen (Table 3). In the prophaseI and telophase I stages of meiosis I in PMCs, a substantial increase ($p < 0.01$) in syncyte cells was observed only in the treated plant with 3% concentration for 24 hours, reaching 0.55%. However, plants treated with 5, 7, and 10 g\l showed a substantial increase ($p < 0.05$) in the percentage of syncyte cells. The seeds exposed to 3% concentration for 24 hours had small number of syncyte cells (0.55%), whereas the seeds subjected to 7% concentration for 24 hours had the most syncyte cells (3.13%). For the treated plants, the effect was generally dose-dependent(table 3).

**Table 3** The frequencies of cytomix and syncyte in PMCs of faba plants exposed to different concentration of naphthalene at different exposure time.

| Treats (g/l) | duration (hours) | total number of PMC observed | PMC with cytomix% | Type of cytomix% | | number of cell showing cytomixis at various meiotic stages% | | | | | | | | Frequency of syncytes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | CC | DF | P-I | M-I | A-I | T-I | P-II | M-II | A-II | T-II | % |
| **0** | 6 | 218 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 ±0.00 |
| | 12 | 210 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 ±0.00 |
| | 18 | 199 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 ±0.00 |
| | 24 | 203 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 ±0.00 |
| **1** | 6 | 211 | 23.12 | 14 | 9 | 0.99 | 0.22 | 0.22 | 0.46 | 0.45 | 0.12 | 0 | 0.78 | 0.00 ±0.00 |
| | 12 | 215 | 26.22 | 15 | 11.22 | 0.87 | 0.99 | 0.99 | 0.55 | 0.5 | 0.34 | 0.45 | 0.8 | 0.00 ±0.00 |
| | 18 | 210 | 27.45 | 14.9 | 12.55 | 0.85 | 0.89 | 0.89 | 0.64 | 0.4 | 0 | 0.6 | 0.8 | 0.00 ±0.00 |
| | 24 | 203 | 29.89 | 18.5 | 11.39 | 1.4 | 1 | 0.66 | 0.99 | 0.9 | 0.7 | 0 | 0.7 | 0.00 ±0.00 |
| **3** | 6 | 198 | 25.57 | 14.67 | 10.33 | 1.34 | 1.86 | 1.82 | 0.5 | 0.23 | 0.59 | 0.68 | 0.98 | 0.00 ±0.00 |
| | 12 | 207 | 30.23 | 18 | 12.23 | 1.45 | 0.99 | 1.99 | 1.2 | 0.64 | 0.35 | 0.6 | 0.66 | 0.00 ±0.00 |
| | 18 | 222 | 30.79 | 17.9 | 12.87 | 1.52 | 0.99 | 1.89 | 1.45 | 0.89 | 0.9 | 0.8 | 0.67 | 0.00 ±0.00 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24 | 215 | 34.45 | 18.56 | 15.89 | 2 | 1.45 | 1.65 | 0.79 | 1.25 | 1 | 0.9 | 1.4 | 0.55 ±0.00 |
| **5** | 6 | 211 | 40.12 | 23.5 | 16.62 | 1.53 | 0.89 | 0.89 | 0.88 | 0.91 | 0.89 | 0.9 | 1.56 | 0.00 ±0.00 |
| | 12 | 216 | 43.33 | 25.68 | 17.65 | 1.98 | 1.34 | 0.84 | 0.97 | 1.2 | 0.9 | 1.21 | 1.67 | 0.62 ± 0.01 |
| | 18 | 223 | 45.75 | 27 | 18.75 | 2.17 | 1.78 | 0.78 | 1.2 | 1.45 | 1.2 | 1.34 | 1.9 | 0.75 ± 0.03 |
| | 24 | 214 | 53.21 | 59.21 | 23.64 | 2.35 | 2.12 | 2.12 | 1.45 | 1.5 | 1.23 | 1.45 | 1.78 | 1.60 ± 0.09** |
| **7** | 6 | 226 | 55.07 | 33 | 22.07 | 1.99 | 0.87 | 1.87 | 1.54 | 0.98 | 1.2 | 1.6 | 1.09 | 1.12 ± 0.32** |
| | 12 | 218 | 57.34 | 35.64 | 21.7 | 1.87 | 0.99 | 0.99 | 1.41 | 1 | 1.67 | 1.93 | 2 | 2.23 ± 0.90** |
| | 18 | 224 | 0 | 0 | 0 | 0.68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.13 ± 1.00 |
| | 24 | 234 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.67 ± 0.32** |
| **10** | 6 | 197 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.10 ± 0.53** |
| | 12 | 204 | 22.3 | 0 | 18.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.16 ± 0.08** |
| | 18 | 214 | 0 | 15.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.60 ± 0.09** |
| | 24 | 189 | 0 | 10.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.89 ± 0.03** |
| **Totals** | | **5081** | **544.8** | **361.36** | **234.21** | **22.99** | **16.38** | **17.6** | **14.03** | **12.3** | **11.09** | **12.46** | **16.79** | **18.42** |

Data are mean of three replicates ± SE. 0.0 = Control group, PI = Prophase I, MI = Metaphase I, AI = Anaphase I, TI = Telophase I, CC cytoplasmic channel, DF= direct fusion; Total no. of PMCs Observed = ~ 200 cells

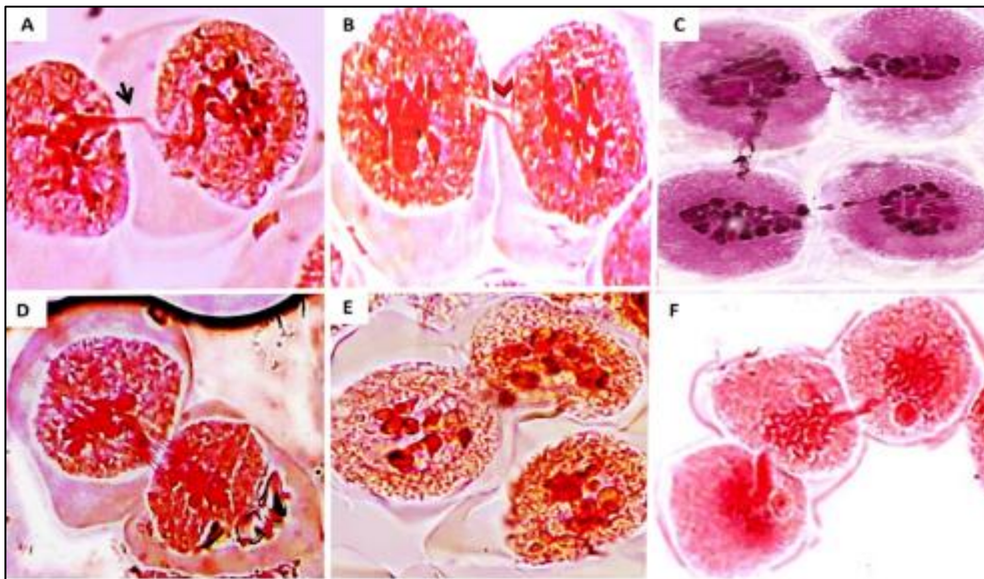


**Figure 3** Cytomixis induced by naphthalene in PMCs of F. bean. A) Cytomixis in PMCs at a different stage, meiotic cells showing migration of part of chromosome. B) PMCs connected through a cytoplasmic channel. C) . C) different stages of meiosis with chromatin and chromosomes could move from donor to recipient cell D) multiple cytoplasmic connections between two PMCs. E) 2 PMCs showing complete fusion F-H) tetrad cells showing migration of chromosomes from donors to recipients I-L) A stained tetrads with Et Br large chromatin strands. M) Different stages of prophase I showing a syncyte.(scale bar: 20μm)

### 3.3. Pollen viability

*3.3.1. NAP promotes pollen size variations*

The impact of NAP-induced cytomix on pollen viability was also investigated.The previous mentioned abnormalities in meiosis resulted in irregular formation of pollen with altered morphology.Pollen grains in the treated group are smaller in size, however there are some unusual patterns that can be seen. A common of Six structural abnormalities were observed (table4)3 classes may be resulted by damage to normal pollen(shrunken, normal empty, large, small, triangular and other forms)and other classes included deformed pollen (with multiple more pores, fused pollen, and pollen with uneven exine, pollen with mitotic figures or other abnormalities) (Table 3). Also the proportion of abnormal pollen varied considerably among individual plants. Some pollen showed very large irregular shape which size which may be resulted from non-reduced gametes. However, the frequency of formation of large pollen grains noticed with high percentage only in plant treated with high NAP concentration (7 and10%)

The most common type of anomaly in pollen morphology was shrunken and normal empty pollen. Shrunken was showed the highest proportion 111.75% of examined pollen and were found 20% (6hrs), 22.3% (12hrs), 23% (18hrs) and 24.8%(24hrs) of 10% treatment. Triangular pollen morphology was rare with 3.2% for all the treated plants. Bicellular pollen grains are typically produced by faba plants (Figures 4, 2A). However, in certain grains, a second pollen mitosis took place, resulting in the generative nucleus being divided and duplicated. The mature tricelluar pollen grains consist of a vegetative nucleus and two sperm cells, which are often arranged in a triangle shape (Figure 4).

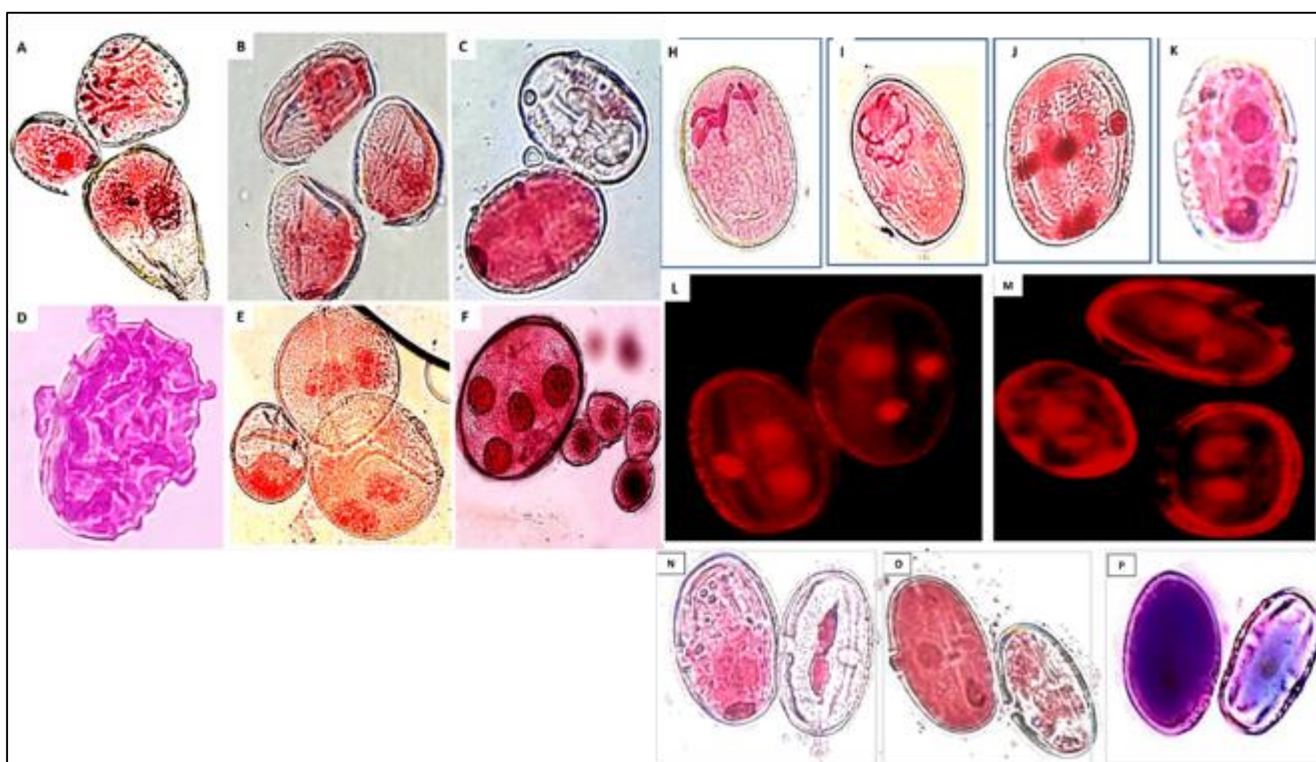


**Figure 4** Representative photomicrographs of the pollen abnormality identified. A) Large irregular shaped. B) shrunken. C) Normal elliptical empty D) deformed grains with multiple pores and pollen tubes. E) heterozygous spherical pollen. F)Multinucleated large pollen. H-I) pollen with mitotic figures. J-K) tricellular pollen. L-M) Tri and bicellular pollen stained with EtBr. O-P) Pollen subjected to different viability staining methods N and O) glyceroacetocarmine stain. P)Alexander's stain. Non viable pollen unstained(red arrows)

*3.3.2. NAP-induced male sterility*

We also assessed the pollen viability(PV)over 6,12, 18 and 24 hrs for both control and treated plants. Using glyceroacetocarmine technique, it was found a no significant difference in pollen fertility between the control plants (more than 98%) and 1 and 3% treatments(PV was estimated to be about 99% in controls. Thus, at 1%, PV had values down to 98% (6 h) to 97 (12 h), 95 (18) and 95.13% (24 h). Also, at 3% of NAP concentration, the pollen viabilityremains in a similar range of the previous treatment, showing95% (6h), 95.4% (12 h), 96% (18h) and 95% respectively (24 h). The maximum pollen sterility was reported at a concentration of 10% incubated at 6 h (74%), 12 h (73.5%), 18 h (70%)

and 24h (68.12%), with the fact that some anthers are totally empty, that shows dependence is exactly here. Similar results obtained using Alexander's stain (Figure 4),PV was estimated to be 100% in controls. However, pollen fertility ranged from 90 to 93% in the 1% treatment. Similar decreasing trends of PV along with increasing dose of NAP were documented in the treated set. However, concentrations of 3%, 5%, 7% and 10% had much clear affect. Hence, mean of PV significantly varied from 90-92%. %, falling between 89-895% at 7% of NAP. 10% NAP- treated plants showed the lowest PV: the average percentage of pollen with normal cytoplasm was from 66 to 71 %. However, both staining methods did not show any significant differences($p \leq 0.05$).

**Table 4** Impact of naphthalene different concentration of pollen morphology and sizes

| Treats g /l | duration (hours) | Normal pollen% | Defective pollen% | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | summary | shrunken | normal empty | Large | small | triangle | other forms |
| 0 | 6 | 100 | 0±00 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 99 | 1±0.04 | 0.35 | 0 | 0 | 0 | 0 | 0.65 |
| | 18 | 100 | 0±0.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 24 | 99.35 | 0.65±0.06 | 0.5 | 0.15 | 0 | 0 | 0 | 0 |
| 1 | 6 | 98 | 2±0.02 | 0.03 | 0.19 | 0.7 | 0.56 | 0 | 0.52 |
| | 12 | 98.5 | 1.5±0.21 | 0.32 | 0.57 | 0.36 | 0.25 | 0 | 0 |
| | 18 | 97 | 3±0.65 | 0.9 | 1.37 | 0.3 | 0 | 0 | 0.43 |
| | 24 | 95 | 5±0.87 | 2.6 | 1.8 | 0.28 | 0 | 0 | 0.32 |
| 3 | 6 | 95 | 5±1.70 | 1.99 | 2 | 0 | 0.22 | 0 | 0.79 |
| | 12 | 93.2 | 6.8±0.02 | 3 | 0.8 | 0.5 | 1 | 0 | 1.5 |
| | 18 | 94 | 6±0.98 | 1.9 | 1.7 | 0.8 | | 0.6 | 1 |
| | 24 | 92 | 8 ± 1.10** | 3.4 | 2.2 | | 0.6 | 0.8 | 1 |
| 5 | 6 | 92.5 | 7.5± 0.54** | 2.98 | 0.72 | 0.33 | 0.8 | 0.5 | 1.5 |
| | 12 | 90.9 | 9.1± 0.89** | 2 | 2.5 | 0.47 | 1 | 0 | 0.83 |
| | 18 | 91.35 | 8.65± 0.98 | 4.4 | 3 | 0.58 | 0 | 0 | 0.67 |
| | 24 | 88 | 12± 3.28** | 4.07 | 3 | 0.75 | 0 | 0.78 | 1.2 |
| 7 | 6 | 88 | 12±2.16** | 5.67 | 4 | 1 | 0.57 | 0 | 0.76 |
| | 12 | 86.7 | 13.3±5.16** | 7.8 | 2.73 | 1.5 | 1 | 0 | 0.96 |
| | 18 | 85 | 15±2.16** | 10 | 4.13 | 0.87 | 0.78 | 0 | 0 |
| | 24 | 83.98 | 16.02±3.10** | 10 | 5.23 | 0.94 | | | 0.55 |
| 10 | 6 | 80 | 20±8.23** | 12 | 6 | 2 | 0 | 0 | 0 |
| | 12 | 77.7 | 22.3± 4.80** | 10.34 | 8.14 | 1.56 | 0.74 | 0.52 | 1 |
| | 18 | 77 | 23±12.3** | 13.5 | 7.5 | 1.45 | 0 | 0 | 2 |
| | 24 | 75.2 | 24.8± 4.60** | 14 | 6.2 | 1.3 | 0.8 | 0 | 2.5 |
| | **totals** | **2177.38** | 222.62 | **111.75** | **63.93** | **15.69** | **8.32** | **3.2** | **18.18** |

### 3.4. Seed efficiency and reproductive success

When various amounts of NAP were applied, there were noticeable differences between the seeded pods on each plant(Table 5). The majority of control pods contained 4 or 5 seeds (Figure 5), whereas the majority of NAP-treated plants had 2 or 3 seeds per pod (Fig. 3A). The highest number of aborted seed number were 45.33, 45.33, 46.67 and 49.33 for the incubation times 6, 12, 18 and 24, respectively, in plants treated with 10%. The second-highest number of aborted seeds number were 32, 40, 43.75 and 44 at 6, 12,18,24 per plant was found 7% treatment, which was statistically similar to the previous aborted seed average (10%). The lowest number of aborted seeded pods per plant was found in control plants (Table5 figure5).We also noted that the aborted seeds have no particular position along the

pods. However, in some cases the aborted seeds were more frequent at the central and the basal parts of the ovaries. Also a few aborted ovule could be seen, less than 3% (figure 5 indicated with the arrows), randomly distributed along the dissected pods. The data presented in table 5 indicate that the smallest pod length for single-seeded pods was 3.5 cm, whereas those for pod with double- or triple-seeded were 6.5 and 9 cm, respectively, compared to 11-13 cm for the control plants. At 7 and 10%, pod length with the 24 hrs was considerably smaller and thinner compared to the other incubation time of 6, 18, and 24hrs. The average pod length decreased with an increase in NAP treatment (5, 7 and 10) from 18 to 24 hrs

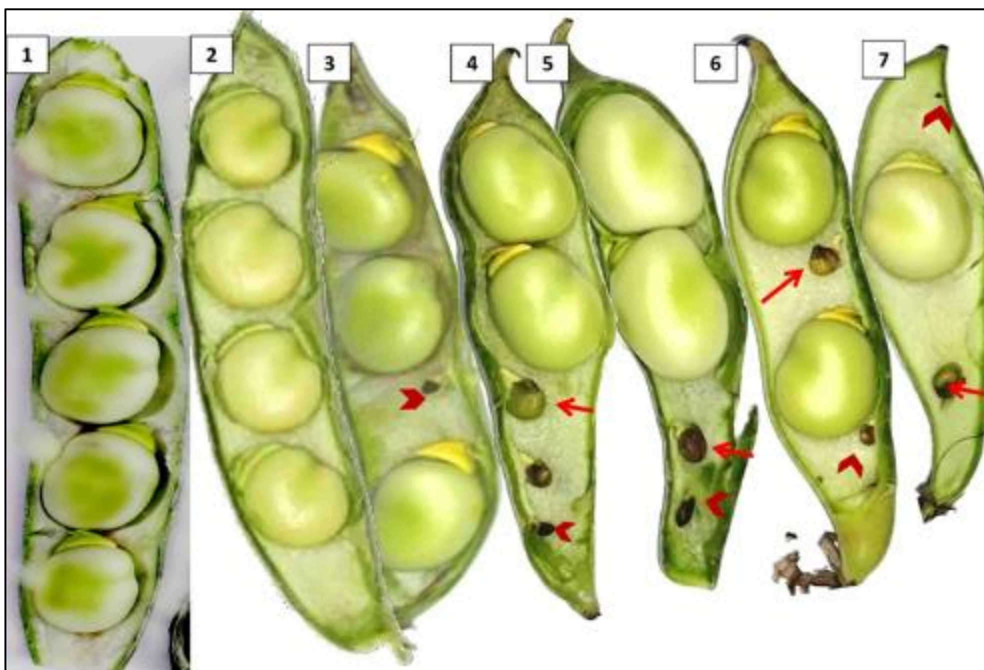


**Figure 5** Faba pods treated with different concentration of Naphthalene showing aborted seeds. 1) pod with 4 and 5 seeds collected from control plants. 3-5 aborted seeds in naphthalene- treated plants (small arrow) aborted ovules (arrow heads)

**Table 5** Means number of pods, seed and ovules in faba plants after treatment with different concentrations of naphthalene at different exposure times

| Treats (g/l) | Duration (hours) | number of pod dissected | no of empty pods | total seeds scored | aborted seed% | aborted ovule |
|---|---|---|---|---|---|---|
| 0 | 6 | 20 | 0 | 98 | 0 | 0 |
| | 12 | 21 | 0 | 100 | 0 | 0 |
| | 18 | 20 | 0 | 98 | 2 | 0 |
| | 24 | 19 | 0 | 94 | 1 | 0 |
| 1 | 6 | 21 | 0 | 100 | 2.76 | 0 |
| | 12 | 20 | 0 | 98 | 2 | 1 |
| | 18 | 20 | 0 | 94 | 6 | 0 |
| | 24 | 21 | 0 | 92 | 12.38** | 1 |
| 3 | 6 | 20 | 0 | 93 | 7 | 2 |
| | 12 | 20 | 1 | 90 | 10** | 0 |
| | 18 | 19 | 1 | 88 | 7.77 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 24 | 18 | 2 | 77 | 15.38** | 1 |
| 5 | 6 | 18 | 3 | 80 | 10** | 0 |
| | 12 | 18 | 2 | 78 | 16.6** | 0 |
| | 18 | 18 | 2 | 70 | 28.57** | 2 |
| | 24 | 18 | 3 | 71 | 21.11** | 1 |
| 7 | 6 | 16 | 3 | 51 | 32** | 0 |
| | 60 | 16 | 4 | 47 | 40** | 3 |
| | 18 | 16 | 5 | 45 | 43.75** | 2 |
| | 24 | 15 | 4 | 42 | 44** | 1 |
| 10 | 6 | 15 | 5 | 41 | 45.33** | 2 |
| | 12 | 15 | 4 | 42 | 45.33** | 2 |
| | 18 | 15 | 6 | 41 | 46.67** | 0 |
| | 24 | 15 | 6 | 41 | 49.33** | 2 |

Seeds and ovule abortions were counted in 15 to 20 pods per treatment. The data are expressed as mean ± SEfollowed ** each row are significantly different at P = 0.05

### 3.5. NAP-induced concentration dependent increase in DNA damage

We also performed the comet assay on faba mature buds obtained from the NAP-treated plants at different incubation times to evaluate DNA damage in pollen nuclei. For the lower NAP concentrations (1 and 3%), there was no significant difference between the exposed groups and the control group for any of the examined incubation times. The Comet assay's measurement of naphthalene genotoxicity in buds and pollen revealed minimal variance; 5% of NAP exhibited a clear DNA damage which is less than 17% at 12hrs. However, there were notable and statistically significant rates of DNA damage as compared to the control group when the NAP was increased to 7 and 10% exhibiting DNA damage of about 30%. This implies that NAP can activate the GST enzyme more when used at higher concentrations (Figure 6and 7).

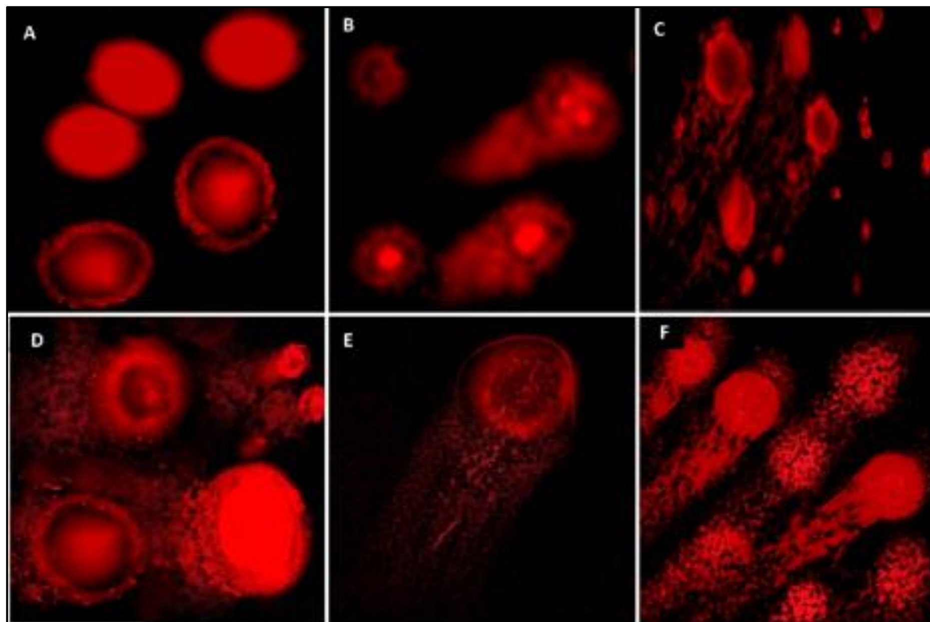


**Figure** 6 Example of comet images Comet Assay of different stages of pollen development, treated with different concentrations of naphthalene and stained with Et Br and observed under fluorescent microscope; A: control, B-F: different stages of pollen development treated with different concentration of NAP. Longer tails indicate more damage. (% tail DNA indicating of extent of DNA damage induced by NAP in male gamete nuclei of faba plants.(scale bar: 20μm)

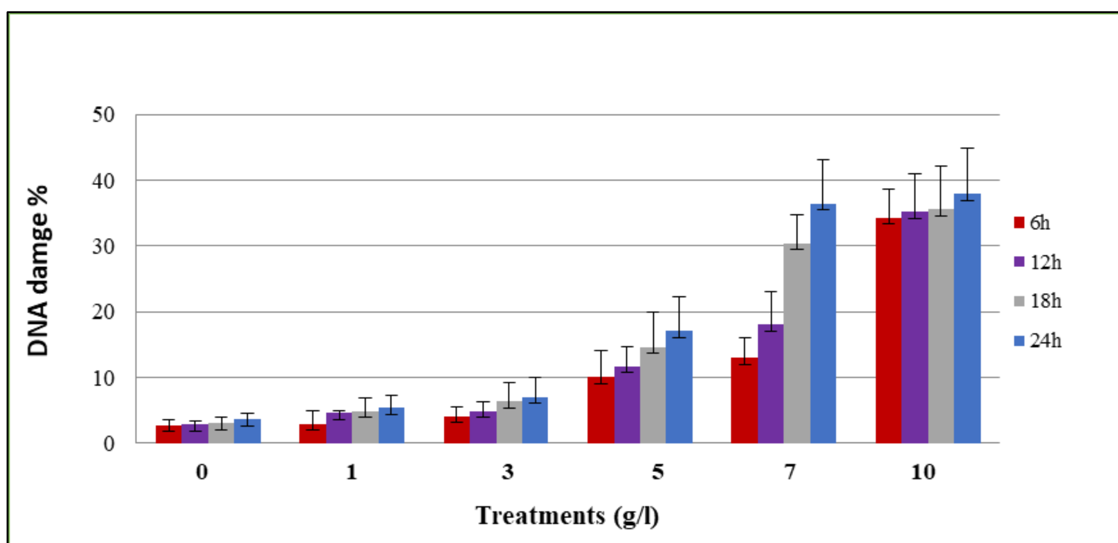


**Figure 7** Concentration dependent increase in DNA damage in mature buds after treatment with naphthalene. Each par representative of three independent replicates (n = 3.) With 75 cells measured per treatments using CaspLap-Comet Assay software. Bars represents slandered deviations

## 4. Discussion

The increasing amount of hazardous chemicals being released into the environment has an effect on the balance of natural ecosystems, which is why many academics and governmental organizations are concentrating on the welfare of living organisms [11]. Because higher plants are useful genetic models for assessing environmental pollutants, these are frequently used in monitoring investigations. Chemical mutagens cause damage to cells, which makes it possible to evaluate genetic end points in cells of different organs, tissues, leaves, roots, and pollen, ranging from point mutations to chromosome aberrations [15,16].

### 4.1. NAP-induced nuclear migration

The cytogenetic analysis of our experiment on faba plants showed that PMCs' cytomixis and syncytes cells, are stimulated by NAP. These phenomena appear to be the result of a cascade of events leading to cytogenetic abnormalities, which impacted the fertilization achievement of male gametogenesis [21, 22]. The meiotic division and its process are significantly impacted by the cytomixis process, which is the movement of chromatin material across PMCs. Originating from the pre-existing plasmodesmata system, cytomictic channels allow for the one-way transport of different organelles and nutrients from more active PMCs to less active ones [23]. Via cytoplasmic connections, cytomictic pathways, and cell wall breakdown, chromosomes and chromatin matter travel among the nearby PMCs [23,24].

The nuclear materials were transferred concurrently via a single or a number of cytomictic conduits. By the end of meiosis I, callose had closed most of the cytomictic transferring channels, which had been found in the early prophase. A number of other studies have reported findings similar to these [25, 26, 27). Researchers hypothesized that cytomixis had a unique effect on microsporogenesis because generative cells can produce polyploidy gametes as parts or the entire nucleus transmitted through cytomictic channels. Cytomictic transmigration and syncytes are generated when the cell walls of adjacent PMCs degrade [22].The donor PMC chromatin material was diminished and drawn near to the location of cytomictic contact during the cytomixis procedure. Then, using cytomictic channels, it was delivered to the recipient cell [26]. These chromatin materials were removed as pyknotic chromosomes based on the results[ 25,28]. Sterile pollen grains and cell degeneration could result from genetically unbalanced cells created by anomalies [22, 27). When the cell membrane breaks down, nearby PMCs engage in cytomictic transmigration, which results in syncytes [22,23, 26].

In our results, entire intact nucleus movement promoted syncytes, which in turn produced large and unreduced pollen. Syncyte appearance has been documented in different plant families include, Asteraceae [28], Fabaceae (22,29], and Poaceae [3], suggesting that it may be a widespread mechanism among angiosperms. At the onset of low-level polyploidy, syncyte formation in diploid plants is essential and contributes significantly to the generation of infraspecific polyploids [28.30]. The simultaneous migrating of chromatin from one PMC to multiple PMCs in faba has also been reported previously[2]. The fate of PMCs involved in cytomixis is determined by partial or full chromatin transfer, which

affects whether the cells create aneuploids, polyploids, or anucleated forms. Prior studies by other researchers [4,25,31] have also observed the formation of these PMCs as a outcome of cytomixis. Since they do not couple with the principal chromatin, the extra chromatin group seen in the PMCs remain separate masses within the cell. Earlier researches showed that mutagenic substances lead to change in the redox state of plant cells [32, 33, 34]. It's probable that NAP causes cytomixis and generates gametes and syncytes with changed chromosomal counts that can be used to develop particular distinctive traits in plants

### 4.2. Effect of NAP treatment on frequencies of chromosomal aberrations and pollen sterilities

Upon comparing the specimens used for testing with the control group, it was observed that the highest meiotic abnormality in the group exposed to NAP was 64.79% while it was 24% in the control group (Table 3). Among these anomalies, sticky chromosome was the most frequently discovered chromosomal defects in our treated plants (Fig. 4). Also,chromosome stickiness and laggards observed with high rates (64.79 and 26.86%) could be caused by genetic and environmental causes[35]. Large concentrations of these abnormal chromosomes are damaging to the vigor and growth of the plants [36].

On the other hand, the low percentage of pollen viability (66.2%) in faba treated plants can be explained by high frequencies of cytomixis, chromosomal stickiness, and other recorded abnormalities. This population's relatively has high occurrence of chromatin mobility at different meiotic stages might be responsible for the low pollen viability observed in it. It can be concluded that meiotic abnormalities significantly affect the meiotic process, thereby reducing pollen viability. Past studies have shown a direct correlation between cytomixis, delayed development, chromosomal stickiness andother anomalies, affecting pollen viability [8.22.37]. The current work reveals that faba plants exhibits varying-sized pollen grains, pollen sterility, and abnormal meiotic activity directly related to cytomixis. Comparable findings on the impacts of cytomixis on meiotic mechanism have been documented in different plants species *Polygonum tomentosum*, B*rassica napus* var. *oleifera* and *B. campestris* var oleifera [31].

### 4.3. Asynchrony in the first and second division enhanced by NAP-treatments

Because of abnormal meiotic processes brought on by unequal spindle activity and chromatin transfer among neighboring PMCs, plants produce faulty sporads and have decreased pollen fertility [8]. Microsporogenesis is disrupted by an abnormal meiotic route, which results in pollen sterility and negatively affects the species' capacity for wild reproduction[8,37]. Knowing the species' breeding habits and the reasons behind meiotic instability is crucial for the conservation and management of rare and endemic plants. [9] reported laggards caused by heavy metals, which is consistent with our findings. One of the cell poles had chromosomal movement failure during anaphase. Due to the random sub-grouping of the chromosome, any deformation or rupture in the spindle may result in the production of monads, dyads, triads and polyads. These aberrant tetrads result in the generation of sterile pollen grains and a drop in the NAP-induced population's meiotic index (83.7%)

The longevity and continued survival of the plant species are closely related to efficient reproduction and ongoing recruitment of new individuals to maintain populations. Based on the current study, the low seed set, which ultimately results in a decline in population size, may be caused by the species' significant meiotic abnormalities. An previous examination of meiotic behavior in certain species indicates that a wide range of anomalies can affect plant fertility or result in complete male sterility [35,38]. However, in the plants under investigation, seed gaps seems to be the consequence of aneuploid or polyploid PMCs as results of cytomixis process. Cytoplasmic interactions between pollen grains were also seen in some research participants. Such connections among low seed set and unreduced pollen have previously been reported by the intergeneric hybrids such Brassica napus var. oleifera and B. campestris var oleifera and V. faba [1,8,9].

### 4.4. NAP causes DNA damage at higher doses

After analyzing the extensive database on NAP genotoxicity that is currently available, it was found that most assays used to evaluate genotoxicity do not show NAP to be mutagenic. Excessive quantities of NAP do not induce mutagenicity in cultured human or bacterial cells, but they do cause toxicity-dependent secondary damage to DNA [39,40]. Secondary damage is indicated by chromosomal breakage in vitro, DNA fragmentation in vivo, chromatid exchange. Chromosome breakage and DNA fragmentation are consistent with a "threshold-related" cytotoxicity mechanism of tumour induction. NAP only produces pulmonary cancers in mice if there are signs of significant cytotoxicity in the NTP bioassay settings, according to very credible scientific assertions. However, the tumors are intimately associated with hyperplasia, prolonged inflammation, cell proliferation, and changes in the metastasis—all The results of the comet assay suggest that male gamete nuclei are not susceptible to the genotoxic effects of any of lower concentration of NAP, even when incubation prolonged to 24 hrs. There was either no difference or a difference that suggested reduced DNA damage

between the treated groups and the control group, according to the statistical analysis of NAP exposure groups. Similar information about the hazardous response to NAP is available in the literature. [42] postulated that indirect mechanisms related to the contamination response can include DNA fragmentation and chromosomal breakage, however these may take longer to show symptoms. In our results, however, a significant DNA damage was observed at higher ANP concentration o NAP with different damage degree according to the variable tail length.A recent evaluation of the extensive already available database on NAP genotoxicity was conducted by the [39,40]. Most of these genotoxicity investigations do not show that naphthalene causes mutations, according to the IARC monograph. [40,41] claims that while naphthalene does not produce mutagenicity in bacteria or mammalian cells cultured at suitable the maximum tolerated dose values, it can cause genotoxicity-dependent secondary damage to DNA at high concentrations. It has been found that free radicals can cause cells' genomes to become unstable. Reactive oxygen species are incredibly unstable and can lead to imbalances in energy metabolism, disruption of the cytoskeleton, and damage to DNA, all of which can result in chromosomal abnormalities [34,35]. It is well known that NAP and other polycyclic aromatic hydrocarbons are potential carcinogenic and genotoxic substances. For example, it has been shown that inhaling NAP causes mice to develop more nasal epithelial tumors in both sexes as well as more bronchial and alveolar adenomas in the females alone [40,42,43]. Double-strand breaks (DSBs) are one of the most significant lesions for preserving genomic integrity among the several types of DNA damage. Because DSBs are so severe, mammalian cells have evolved intricate signaling pathways in reaction to them. On the other hand, the primary initiators of carcinogenesis are thought to be unrepaired or improperly repaired double-strand breaks (DSBs), which typically occur when the error-prone non homologous end-joining repair pathway is used[42]of which are signs of cytotoxicity [41].

## 5. Conclusion

In conclusion: our results clearly exhibited that that increased syncyte manifestation, cytomixis, and pollen fertility in PMCs generated on V. faba following NAP treatments. By using comet assay, the results of the current study showed how NAP induced DNA damage at higher concentrations. Our results also concluded that naphthalene is capable of inducing NAP resulted in altering the number of chromosomes, which can be further used in breeding initiatives to generate favorable genetic variations.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

All authors declare that they have no conflicts of interest.

### *Author contributions*

HAK and NSE designed the study and wrote the manuscript. HAS performed the experiments.

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