**Original Research Article**

**EFFECT OF ENDOPHYTIC FUNGAL EXTRACT OF *DREGEA VOLUBILS* (L.F.) BENTH. ON DIVIDING CELLS OF ALLIUM SATIVUM, TELANGANA STATE, INDIA**

**ABSTRACT**

In this study, we looked at the mitotic cell divisions in dividing root tip cells of Allium sativum to see whether crude extracts of three endophytic fungal species may be mutagenic. Observations were made about the following. The mitotic index (MI) and other abnormalities were both significantly impacted by crude Penicillium rubrum extracts. As the treatment time and concentration of the crude extract increased, MI dropped.These extracts induced different types of abnormalities. Among this extreme fragmentation in metaphase were found to be more common. Fragmentation of chromosomes was also a common aberration in telophase. Crude extracts of *Phyllostictacapitalensis* have also exhibited a significant effect on MI and mitotic aberrations. Three types of aberrations viz, stickiness, breakages and extreme fragmentations were recorded in metaphase. Fragmentation type of aberrations was noticed during telophase. The total percentage of abnormalities increased upto nearly 30% of crude extracts concentration. Crude extracts of *Phyllostictafallopiae* have also shown significant effect on MI and chromosomal aberrations. In this case, metaphase stickness, breakages and extreme fragmentation type of aberrations were observed. Fragmentation and laggards were recorded during anaphase. A comparison between the effects of extracts of three endophytic fungi revealed that there is a slight difference, however not significant, in inducing the abnormalities. All of them induced almost same type of abnormalities. There is also not much difference with MI.

**Keyword:** *Allium sativum, Dregeavolubilis,*Endophytic fungi*,* Mitotic index,*Penicillium rubrum,Phyllostictacapitalensis,Phyllostictafallopiae*.

**INTRODUCTION**

Fungal endophytes may have a wide range of interactions with the plants that they inhabit, including symbiosis, mild pathogenicity, antagonism, and mutualism (Schulz and Boyle 2005, Arnold 2007). Zhou and Hyde (2001) and Cohen (2006) identify host-specificity, host-recurrence, host-selectivity, and host preference as characteristics of endophyte interactions. When microbes are limited to one host species or a small number of closely related ones, this kind of association is known as host-specificity and is seen in endophyte-plant interactions. The specificity of the relationship between the host and the endophytes implies complex metabolic processes (Holliday 1998, Strobel and Daisy 2003). It has been shown that certain endophytic fungi can produce compounds with various medicinal uses, such as those against bacteria, viruses, inflammation, and tumors. These compounds can be derived from alkaloids, steroids, flavonoids, terpenoids, and other unique structural types (Guo et al. 2008, Yu et al. 2010). Aly et al. (2011) and De Souza et al. (2011) have provided a thorough and current review of compounds derived from endophytic fungi spanning from 1995 to 2011. Their work encompasses aspects of botany, phytochemistry, pharmacology, and toxicology, while also addressing potential trends and outlining future research directions for endophytes.

Apocynaceae family member Dregeavolubilis, more often known as Jutiki, is a woody climber that grows in the hottest regions of India. It grows wild over the whole state of Telangana and is used for traditional medicine, herbal remedies, and as an antioxidant.

Traditionally, *D. volubilis* is used alone or in combination with other medicinal plants and exhibits antibacterial, antifungal, antitumor (Molisha*et al*. 2009), activity and is hepatotoxicity (Tennekoon*et al.* 1991), and used to treat snake bites, headache, emetic, eye diseases, abscess, rheumatic pain, cough, severe cold, piles, leucoderma, asthma and urinary discharge (Kirtikar and Basu 1985).The therapeutic use of the plant has been shown in almost every region of it. Among the various herbal and folk remedies, the leaves are well-liked for their pharmacological effect, which makes them useful for treating boils and abscesses (Subapriya and Nagini 2005; Sahu et al. 2002). The medicinal properties of plants have been recognized by traditional healers for a very long time (Pullaiah. 2002). Isolation and characterization of twelve polyhydroxy C/D cis pregnane glycosides from D. volubilis were reported by Udhyasankar et al. (2012).Two chemicals isolated from D. volubilis's stem and flowers have been shown to be effective against Enrlich's ascites carcinoma, according to a study (Sahu et al. 2002). It is possible that endophytic fungi species that are connected with the host plant are responsible for producing these active metabolites. In terms of frequency with D. volubilis, P. rubrum, P. capitalensis, and P. fallopiae rank highest. To determine the potency of mutagens, one of the most reliable indices is cytological study with regard to mitotic or meiotic behavior. Consequently, the majority of mutation research include inquiries into mitotic aberrations and the genetic effects of these abnormalities. It also gives us a good idea of how to measure plant susceptibility to various mutagens. Because of their big, monocentric chromosomes, Allium sativum and Allium cepa are considered to be good models for studying environmental mutagenesis. Numerous employees used these techniques to assess the mutagenicity of different substances. Using a modified A. cepa test, Nielsen (1993) screened complicated combinations for genotoxicity. Abdelmigid(2009) utilized V. faba beans as an indication to investigate the genotoxic impact of synthetic colors used as food additives, whereas Ma et al. (1995) performed a similar test with a micronucleus assay to evaluate the clastogenic effect of environmental contaminants. The findings of environmental pollution and cytotoxicity assessments using the same test method are consistent with those of in-vivo animal cytotoxicity studies (Vicentini et al. 2001). Two separate studies on Allium (A. cepa and A. sativum) found that avenoxan was genotoxic (Teixeira et al., 2003; Tartar et al., 2006). As part of their study on the mutagenicity of silvarnanoparticles, Kumari et al. (2009) found the most prevalent chromosomal abnormalities, including c-metaphases, sticky chromosomes, chromosome breaks and losses, bridging anaphases, multipolar anaphases, micronucleated cells, and binucleated cells.

There are few studies on effect of fungal extracts on cytotoxicity (Badr A 1986 and Gaulden ME 1987)However, there are no such studies as cytotoxic effect of fungal extracts isolated from *D. volubilis*. Hence, an attempt is made to study the effect of fungal extracts of three fungal species on the dividing cells of *Allium sativum*.

**MATERIALS AND METHODS**

**Collection of plant material**

From Dregeavolubilis plant from various geographic sites in Warangal in Telangana state, India (Warangal 17 59N, 79 35E 263m height), tissue samples of leaves (59) were gathered winter season. Carefully selected for sample were healthy, six to twelve year old mature leaf portions. Samples were kept at 4º C in a on thermocol ice box throughout transit and handled 48 hours in Ziploc bags.

**Isolation of endophytic fungi**

In order to eliminate any dirt or debris, the samples of D. volubilis leaves were first washed in sterile water for a few minutes after being rinsed in tap water (Selvanathan et al. 2011). In all, 885 segments were chosen for the purpose of sampling various leaves. The midrib and lateral sections of mature, healthy leaves were chopped into pieces about half an inch to an inch wide. From Dobranic et al. (1995). To sanitize their surfaces, the leaf segments were submerged in 70% ethanol for 60 seconds, then in 4% sodium hypochlorite for 180 seconds, once again in 75% ethanol for 30 seconds, and finally in sterile distilled water for 10 seconds.Approach proposed by Goveas et al. in 2011. To prevent the samples from becoming too wet, they were placed on sterile filter paper. After the segments were steriled on the outside, they were put on agar plates using parafilm. Streptomycin at a concentration of 120 mg/liter was added to the potato dextrose agar medium.

**Identification**

In a 24-hour light/dark cycle, each Petri dish with four segments was maintained at a temperature of 27± 2 ºC (Suryanarayanan et al. 2003). The development of endophytic fungal colonies from the plant sample was checked daily after 4 to 6 days by monitoring the petri-dishes. For future research, the sample pieces were transferred to new tubes containing potato dextrose agar (PDA) slants to preserve the fungal isolates. The tubes were then refrigerated at 4°C. The shape of each fungal colony was documented. Lactophenol cotton blue was used to create microscope-ready slides of fungal colonies. The features of mycelia and spores were documented. Fungi were identified using photomicrographs captured under a fluorescent microscope and referenced from established manuals (Barnett and Hunter 1998).

**Fungal culture and extraction**

Radji et al. (2011) provided the procedure that was used to manufacture the fungal crude extract. Two hundred and twenty milliliters of potato dextrose broth was added to 500 milliliter Erlenmeyer flasks before the endophytic fungus isolates were inoculated. 200 grams of potato extract, 20 grams of dextrose, and 1000 milliliters of distilled water were mixed and left to incubate at 27± 2 ºC with periodic shaking for 14 days. The fungal broth culture was prepared for extraction by filtering it with whatman no. 1 filter paper, which yielded a clear filtrate and mycelia. We used a rotary evaporator to evaporate the liquid supernatant under decreased pressure after extracting it with an equivalent amount of organic solvents (Methanol). For the first assessment, the unrefined extracts were used. Researchers looked at how Allium sativum dividing cells responded to crude extracts of certain fungal endophytes by measuring their mitotic index (MI).

**Cytototoxicity effect of fungal endophytes**

The aim of this Cytotoxic work is to examine the effect of fungal crude extracts of *Penicillium rubrum,Phyllostictacapitalensis and Phyllostictafallopia* on the mitotic cell division of the root tip cells of *Allium sativum*.

**Preparation of test sample:** The crude extracts of Penicillium rubrum and Phyllosticta sp. were selected, and the following concentrations of these extracts were prepared: 100, 150, 200, and 250 µg/ml. This study considered Allium sativum with 80 cloves. Each clove was permitted to develop roots by immersing them in beakers filled with water at room temperature. Following a 72-hour period, the roots were placed into beakers with varying concentrations of fungal extracts, where the garlic roots were subsequently immersed in the solutions for an additional 24 hours. Root tips of the garlic sample, approximately 1 cm in length, were collected into vials between 8:30 AM and 9:30 AM. The processes of pretreatment, fixation, hydrolysis, squashing, staining of cells, and slide preparation were conducted in accordance with the methodology established by Akinyele (2007).

**Pretreatment:** Before being placed in the pretreatment solution for three hours, the root tips of Allium sativum that were taken from each treatment were placed in specimen vials that contained paradichlorobenzene.

**Fixation:** The pretreated root tips were then taken out of the paradichlorobenzene solution, washed three times with distilled water to eliminate any remaining paradichlorobenzene, and finally fixed in a mixture of one-third glacial acetic acid and three quarters 100% ethanol. The purpose of the fixative was to destroy the root cells while maintaining their original state to prevent the leaching of cell contents. All vials were placed in the refrigerator at 4°C for 24 hours after the specimen was appropriately labeled.

**Hydrolysis:** After a day in the fixative, the root tips were taken out and given a good rinsing in distilled water for a couple of minutes before hydrolysis. A 10% solution of ammonium chloride was then added to the rinsed root tips and let to sit at room temperature for 10 minutes. The root tips were loosened by hydrolysis.

**Squashing and staining:** After the root tips had been hydrolyzed, they were carefully rinsed with distilled water and set on a fresh glass slide. Squeezing the root tips with the wider, flat end of a cylindrical search needle produced a turbid solution after applying two drops of aceto-oricin stain and covering them with cover slip. The slides were then warmed slightly using a spirit lamp's flame. The extra stain was squeezed out of the slides by gently pressing them between two layers of folded filter paper. To seal the cover slip and keep air out, we used DPX (Dibutylphthalate polystyrene xylene). In this way, three labelled slides were made for every treatment.

**Observation of cell division and chromosomes:** The slides were placed on the microscope slide mount and examined using the fluorescent light. When examining the slides, the 10x, 20x, and 40x objectives were used. The photomicrograph was used to capture images of various mitotic phases and chromosomal aberrations. Also documented were the counts of various mitotic phases.

**Data analysis**

Ten counts of each were acquired under the following directions from ten different slides from each concentration. There were found total 3500–4000 cytologically changed cells. Research revealed aberrant cells, count of interphase cells, prophase cells, metaphase cells, anaphase cells, telophase cells.

***Mitotic Index (MI)***

Calculating the proportion of abnormal cells Using the following formula suggested by Malode et al. (2012), the Mitotic Index (MI) for the cells subjected to various concentration of fungal crude extracts was computed.

*Mitotic Index (MI)100*

The count of dividing cells was determined by the sum of prophase, metaphase, anaphase, and telophase stages.

**RESULTS AND DISCUSSION**

This work aimed to assess, on mitotic cell division in developing root tip cells treated at different doses, the possible mutagenicity of crude extracts of three species of endophytic fungus.

**Effect of *Penicillium rubrum* crude extracts on Mitotic Index (MI) and mitotic divisions on dividing cells of *Allium sativum***

A critical study of the table 1 reveals that the crude extracts of endophytic fungus *P. rubrum* have shown a definite effect both on mitotic index and different abnormalities at different stages.(Table.1, Text Fig. 1and Plate 1). The effect varied both with the concentration of the effect and duration of the treatments. The percentage of mitotic index (MI) indicates the percentage of dividing cells. MI decreased with increase in treatment duration in every concentration. Similarly, MI decreased in with increase in concentration. Different mitotic abnormalities were induced with the treatments of crude extract of *P. rubrum.* Metaphase abnormalities included stickiness, breakages and extreme fragmentations. According toJayabalan and Rao (1987) suggested stickiness might be due to disturbances in cytochemically balanced reactions. Breakage type of abnormolites found to be more than the stickiness and extreme fragmentations. The percentage of all these abnormalities increased both with treatment duration and concentration. At Anaphase stages two type abnormalities i.e. fragmentation and laggards were recorded. Fragmentation types of abnormalities were induced more than the laggards. These anomalies also escalated with the concentration and duration of the medication. The occurrence of chromosomal bridges may result from stickiness or the creation of dysenteric chromosomes owing to breakage and reunion (Dempong and Maxwell 1973).

Fragmentation of chromosomes was a common abnormality in telophase stage. This type of abnormalities also increased both with concentration of crude extract and duration of treatment. Among different types of abnormalities, extreme fragmentations in metaphase were observed to more. Least percentage abnormalities those of laggards in anaphase and fragmentation in telophase.

**Effect of *Phyllostictacapitalensis* crude extracts:**

Crude extract of *Phyllostictacapitalensis* have also exhibited profound effect on mitotic index and mitotic abnormalities. (Table. 2 and Text Fig. 2).Both effects markedly intensified with the extract concentration and treatment time. The proportion of MI escalated with the concentration and duration of therapy. The importance is increasingly apparent with increased treatment duration. Three forms of abnormalities, including stickiness, breakages, and severe fragmentation, were seen during metaphase. Nevertheless, the stickiness problem is less prevalent than the other two forms of anomalies. Numerous studies documented fractured cells, and achromatic lesions were also seen. Stickiness, a prevalent abnormality during metaphase, may result from the depolymerization of nucleic acids induced by carcinogenic therapies or from partial dissociation of nucleoproteins and alterations in their organizational patterns (Evans 1962).

However, these abnormalities increased with both concentration and duration Fragmentation and laggards’ type abnormalities were observed in anaphase stage of mitosis. But fragmentations were found to be more than the laggards. In telophasic stage, fragmentation types of abnormalities were induced. As in the case of other abnormalities, these were found to increase with the concentration of crude extract and duration of treatment. The total percentage of abnormalities increased up to nearly 30%. Least number of abnormalities was those of telophase fragmentation.

**Effect of *Phyllostictafallopiae* crude extracts:**

Present are the findings about the influence of crude extract of P. fallopiae on dividing cells of Allium sativum. Table. 3 and Text Figure 3. A detailed examination of the data shows that at many phases of mitosis the extracts clearly affected MI and cell division aberrations. Still, the impact varied depending on the extract concentration and treatment length. MI dropped with increasing concentration and length of time. Still, the concentration has greater effect than the time. Observed were metaphase stickiness, breakages, and severe fragmentation type of anomalies. Recording more than stickness and fragmentation were breakage anomalies. Concentration of the extracts and length of therapy affected all these irregularities. Abnormal spindle formation and inability of chromosomal mobility help to explain lagging chromosomes described by Abrham and Rajlakshmy in 1989. Anaphase saw recording of laggards and fragmentation. Still, fragmentation kinds of irregularities were noted more than the laggards. Treatment duration and concentration raised both these anomalies. There were also observed telophase fragmentations. Their concentration increased with the longer treatment times and higher concentration. The overall proportion of anomalies also changed. Comparatively, the impacts of three endophytic fungus show that while there is a little variation in producing the anomalies, that difference is not statistically significant. From 24.72 to 29.39, the proportion of overall anomalies ranges from lower number for P. rubrum to higher value for P. capitalensis. Every one of them caused the same kind of anomalies. Also not much differs with MI.

**CONCLUSION**

In the present investigations, an attempt was made to evaluate the potential mutagenicity of crude extracts of three species of endophytic fungi through observation of mitotic cell divisions of *Allium sativum.* Crude extracts of *P. rubrum* had a definite effect both on mitotic index (MI) and on different aberrations. MI decreased with increase in treatment duration and concentration of crude extract. These extracts induced different types of abnormalities. Among these extreme fragmentations in metaphase were found to be more common. Fragmentation of chromosomes was also a common aberration in telophase. Crude extracts of *P. capitalensis* have also exhibited a significant effect on MI and mitotic aberrations. Three types of aberrations viz. stickiness, breakages and extreme fragmentations were recorded in metaphase. Fragmentation type of aberrations was noticed during telophase. The total percentage of abnormalities increased up to nearly 30% of crude extracts concentration. Crude extracts of *P. fallopiae* have also shown significant effect on MI and chromosomal aberrations. In this case, metaphase stickness, breakages and extreme fragmentation type of aberrations were observed. Fragmentation and laggards were recorded during anaphase. A comparison between the effects of extracts of three endophytic revealed that there is a slight difference, however not significant, in inducing the abnormalities. All of them induced almost same type of abnormalities. There is also not much difference with MI.

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**Table 1: Effect of *Penicillium rubrum* crude extracts on Mitotic Index (MI) and mitotic divisions on dividing cells of *Allium sativum***

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration (ug/ml)** | **Duration (in min)** | **Mitotic Index**  **(%)** | **Metaphase** | | | **Anaphase** | | **Telophase fragmentation**  **(%)** | **Total (%)** |
| **Stickness**  **(%)** | **Breakages**  **(%)** | **Extreme fragmentations (%)** | **Fragmentation**  **(%)** | **Laggaards**  **(%)** |
| **Control** |  | 11.42 |  | | | | | | |
| **100** | 60 | 8.20 | 0.10 | 1.55 | 1.35 | 1.40 | 0.40 | 0.48 | 5.28 |
| 120 | 7.15 | 0.30 | 1.99 | 1.50 | 1.87 | 0.75 | 0.79 | 7.2 |
| 180 | 6.24 | 0.45 | 2.11 | 1.70 | 2.30 | 1.00 | 1.09 | 8.65 |
| 240 | 5.98 | 1.20 | 3.20 | 2.55 | 3.90 | 1.05 | 1.49 | 13.39 |
| **150** |  | | | | | | | | |
| 60 | 9.20 | 0.75 | 1.45 | 1.08 | 1.11 | 0.29 | 0.16 | 4.84 |
| 120 | 8.09 | 0.90 | 1.80 | 1.34 | 2.14 | 0.36 | 0.67 | 7.21 |
| 180 | 6.30 | 1.55 | 2.00 | 2.25 | 2.20 | 1.50 | 1.00 | 10.5 |
| 240 | 5.40 | 1.96 | 2.75 | 3.15 | 3.88 | 2.35 | 1.98 | 16.07 |
|  | | | | | | | | |
| **200** | 60 | 6.50 | 1.00 | 1.20 | 2.10 | 1.12 | 0.41 | 0.28 | 6.11 |
| 120 | 6.00 | 1.27 | 2.54 | 2.68 | 2.10 | 1.35 | 1.10 | 11.04 |
| 180 | 5.68 | 2.00 | 3.00 | 3.11 | 3.10 | 1.55 | 1.78 | 14.54 |
| 240 | 5.13 | 2.65 | 3.22 | 4.15 | 5.15 | 2.12 | 2.18 | 19.47 |
|  | | | | | | | | |
| **250** | 60 | 5.66 | 3.66 | 1.02 | 2.20 | 2.21 | 3.00 | 1.54 | 13.63 |
| 120 | 4.15 | 4.00 | 1.15 | 3.55 | 3.27 | 4.16 | 1.94 | 18.07 |
| 180 | 3.40 | 4.50 | 1.65 | 3.15 | 4.00 | 4.68 | 2.24 | 20.22 |
| 240 | 3.00 | 5.22 | 2.20 | 4.50 | 4.12 | 5.92 | 2.76 | 24.72 |

**Table 2: Effect of *Phyllostictacapitalensis*crude extracts on Mitotic Index (MI) and mitotic divisions on dividing cells of *Allium sativum***

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration (ug/ml)** | **Duration (in min)** | **Mitotic Index (%)** | **Metaphase** | | | **Anaphase** | | **Telophase fragmentation**  **(%)** | **Total (%)** |
| **Stickness (%)** | **Breakages (%)** | **Extreme fragmentation (%)** | **Fragmentation (%)** | **Laggaards**  **(%)** |
| **Control** |  | 12.14 |  | | | | | | |
| **100** | 60 | 10.5 | 0.1 | 1.02 | 1.34 | 1.33 | 0.36 | 0.55 | 4.71 |
| 120 | 9.9 | 0.5 | 1.19 | 1.05 | 1.12 | 0.85 | 0.75 | 5.21 |
| 180 | 8.54 | 0.8 | 2.11 | 1.06 | 2.34 | 1.01 | 0.87 | 7.76 |
| 240 | 7.5 | 0.15 | 3.25 | 2.55 | 4.23 | 1.25 | 0.99 | 13.66 |
| **150** |  | | | | | | | | |
| 60 | 9.35 | 0.75 | 2.42 | 1.88 | 1.06 | 0.45 | 0.55 | 7.11 |
| 120 | 8.22 | 0.66 | 2.56 | 2.23 | 2.01 | 0.86 | 0.75 | 9.07 |
| 180 | 7.89 | 1.05 | 2.89 | 2.05 | 2.33 | 0.99 | 0.99 | 10.3 |
| 240 | 6.65 | 1.65 | 3.75 | 3.95 | 4.92 | 1.35 | 1.25 | 16.87 |
|  | | | | | | | | |
| **200** | 60 | 7.51 | 1.12 | 2.44 | 2.23 | 1.77 | 0.8 | 0.99 | 9.35 |
| 120 | 7.00 | 1.32 | 2.11 | 2.00 | 2.56 | 1.22 | 1.23 | 10.44 |
| 180 | 6.69 | 2.02 | 3.28 | 3.01 | 3.87 | 1.55 | 1.54 | 15.27 |
| 240 | 6.23 | 2.13 | 3.89 | 5.22 | 5.09 | 1.94 | 1.08 | 19.35 |
|  | | | | | | | | |
| **250** | 60 | 5.28 | 1.22 | 3.43 | 3.00 | 3.45 | 1.55 | 1.00 | 13.65 |
| 120 | 5.15 | 2.43 | 4.34 | 4.87 | 4.12 | 1.45 | 2.56 | 19.77 |
| 180 | 4.35 | 2.55 | 4.23 | 5.01 | 4.65 | 2.33 | 2.09 | 20.86 |
| 240 | 4.00 | 1.08 | 6.75 | 8.12 | 7.92 | 2.51 | 3.01 | 29.39 |

**Table 3: Effect of *Phyllostictafallopiae* crude extract on Mitotic index (MI)and mitotic division of dividing cells of *Allium sativum***

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration (ug/ml)** | **Duration (in min)** | **Mitotic Index**  **(%)** | **Metaphase** | | | **Anaphase** | | **Telophase fragmentation**  **(%)** | **Total (%)** |
| **Stickness**  **(%)** | **Breakages**  **(%)** | **Extreme fragmentation (%)** | **Fragmentation (%)** | **Laggaards**  **(%)** |
| **Control** |  | 12.10 |  |  |  |  |  |  |  |
| **100** | 60 | 9.20 | 0.09 | 0.55 | 0.35 | 1.10 | 0.20 | 0.18 | 2.47 |
| 120 | 8.15 | 0.20 | 0.99 | 0.57 | 1.57 | 0.75 | 0.59 | 4.67 |
| 180 | 7.24 | 0.25 | 1.11 | 1.06 | 2.10 | 1.10 | 0.99 | 6.61 |
| 240 | 8.98 | 1.28 | 2.20 | 1.55 | 3.90 | 1.55 | 1.19 | 11.67 |
| **150** |  | | | | | | | | |
| 60 | 9.20 | 0.55 | 1.44 | 1.40 | 1.61 | 0.49 | 0.66 | 6.15 |
| 120 | 9.00 | 0.60 | 1.85 | 1.94 | 2.14 | 0.96 | 0.97 | 8.46 |
| 180 | 8.55 | 0.95 | 2.30 | 2.15 | 2.30 | 1.30 | 1.19 | 10.19 |
| 240 | 7.50 | 1.06 | 2.75 | 2.95 | 4.88 | 1.75 | 1.98 | 15.37 |
|  | | | | | | | | |
| **200** | 60 | 7.20 | 1.11 | 2.20 | 2.00 | 1.77 | 0.81 | 0.98 | 8.87 |
| 120 | 6.55 | 1.20 | 2.54 | 2.45 | 2.60 | 1.15 | 1.20 | 11.14 |
| 180 | 5.78 | 2.00 | 3.12 | 3.10 | 3.50 | 1.75 | 1.88 | 15.35 |
| 240 | 5.23 | 2.05 | 3.32 | 4.22 | 5.65 | 2.22 | 2.08 | 19.54 |
|  | | | | | | | | |
| **250** | 60 | 4.66 | 1.12 | 3.20 | 3.21 | 3.33 | 1.44 | 1.77 | 14.07 |
| 120 | 4.15 | 1.55 | 3.85 | 3.67 | 4.11 | 1.84 | 2.50 | 17.52 |
| 180 | 3.50 | 1.95 | 4.15 | 4.10 | 4.65 | 2.14 | 2.91 | 19.9 |
| 240 | 3.22 | 2.00 | 5.50 | 5.12 | 6.92 | 2.66 | 3.10 | 25.3 |

**Figure 1: Effect of *Penicillium rubrum* crude extracts on Mitotic Index (MI) and mitotic divisions on dividing cells of *Allium sativum***

**Figure 2**:**Effect of *Phyllostictacapitalensis*crude extracts on Mitotic Index (MI) and mitotic divisions on dividing cells of *Allium sativum***

**Figure3: Effect of *Phyllostictafallopiae* crude extract on Mitotic index (MI)and mitotic division of dividing cells of *Allium sativum***

|  |  |  |
| --- | --- | --- |
| **C:\Users\admin\Desktop\aruna\seq0753 (6).tif** | **C:\Users\admin\Desktop\aruna\seq0739 (4).tif** | **C:\Users\admin\Desktop\aruna\seq0757 (4).tif** |
| **A** | **B** | **C** |
| **C:\Users\admin\Desktop\aruna\seq0763 (3).tif** | **C:\Users\admin\Desktop\aruna\seq0707 (3).tif** | **C:\Users\admin\Desktop\aruna\seq0742 (5).tif** |
| **D** | **E** | **F** |
| **C:\Users\admin\Desktop\aruna\seq0746 (3).tif**  G | **C:\Users\admin\Desktop\aruna\seq0705 (4).tif**  H | **C:\Users\admin\Desktop\aruna\seq0733 (6).tif**  I |

**Plate1**: Photomicrographs of the treated *Allium sativum* root tips cells showing different mitotic stages and aberrations. A. Prophase B. Metaphase C. Anaphase D. Telophase E. Sticky metaphase F. Polar deviation G. Abnormal distribution of chromosomes H. chromosomal bridge I. Nuclear lesion.