Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2342

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **4 (4):** 114-122 (2016)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Effect of Organophosphorus Insecticide, Malathion on the Division of Meristems of *Allium cepa* L.

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Received: 25.07.2016 | Revised: 3.08.2016 | Accepted: 5.08.2016

ABSTRACT

The objective of the present work is to study the effect of an organophosphorus insecticide (Malathion) on the division of meristems of Allium cepa. l. Fresh healthy onion bulbs were treated with different concentration of Malathion from 0.1% - 0.9%. Treated bulbs were sliced into transverse section and mitotis slides were prepared by Acetocarmine hydrolysis technique to observe the changes in mitotic phases under a microscope. A series of mitotoxicity was observed under the influence of Malathion. The abnormal mitotic phases can be clearly observed in the disturbed metaphase and anaphase and shrinkage of anaphase chromosomes. In, 0.1%, 0.2% and 0.5% concentration the chromosomes got separated and loosened, clearly avoiding the pair formation and in some instances, spindle formation was inhibited. The maximum mitotic division was observed in Treatment 1 (0.1%) and Treatment (0.2%). The initial mitotic depression was observed in Treatment 4 (0.4% concentration) which gradually increased with increasing concentration. Highest mitotic depression was observed in Treatment 9 (0.9%) with a Mitotic Index (MI) of 11%. Malathion can be mitotoxic for plant causing aberration in different mitotic phases and which can arrest cellular growth and leads to senescence.

Key words: *Malathion, Allium cepa L., Mitotoxic Organophosphorus, Mitosis, Metaphase*

INTRODUCTION

Onion (*Allium cepa L.*) is a vegetable which is widely cultivated species of genus *Allium*[6,7]. Onions are grown chiefly as "green onions"[9] or "dry bulb"[3]. Harvesting of the green bunching onions is done before the bulbs get developed and the leaves should remain green whereas, dry bulb onions are harvested only when they have matured and the leaves have died[13]. Onions have a wide range of applications in the field of agriculture, biomedical and food. They are widely used as ingredients in various types of culinary practices (commonly used in salads and as flavoring agents in the preparation of curries, stews, soups, etc.)[8]. Onion juice is used in various intestinal and gastric disorders and also against fighting tuberculosis, typhoid fever, abnormal accumulation of liquid in cellular tissue diabetes, caught, rheumatism, skin diseases and insect bites[14,19].

Cite this article: Gogoi, P., Das, S., Das, S. and Khan, M.Z.A., Effect of Organophosphorus Insecticide, Malathion on the Division of Meristems of *Allium cepa* L., *Int. J. Pure App. Biosci.* **4(4):** 114-122 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2342

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Malathion [S - (1, 2-di-carb-ethoxy-ethyl) - O, O -di-methyl-di-thio-phosphate] - a non-systemic broad spectrum Organophosphorus (OP) pesticide, is also better cited as Carbophos, Maldison and Mercaptothion[1]. Malathion is a yellow to brown liquid with a skunk or garlic-like odor. It dissolves slightly in water. It may damage metal and some forms of plastic and rubber. It is found in various states like - Wettable Powder (WP), Pressurized Liquid (PL), Emulsifiable Concentrate (EC), dust etc[27]. Some common Malathion products used for agricultural and domestic settings are Acme Malathion, Agrisect, Atrapa, Black Leaf Malathion spray, Bonide, Clean Crop Malathion, Eliminator, Fyfanon, Gowan Malathion Dust etc[26]. Malathion is suited for the control of sucking and chewing insects of fruits and is used in veterinary medicine[20]. It has been effective in the control of pests on vegetables, field crops, fruits, agriculture, commercial extermination, fumigation, domestic animals, and veterinary practices[1]. Malathion kills insects by disruption the nervous system. It does this by inhibiting an enzyme called cholinesterase. Malathion also affects humans but insects are more susceptible to it than mammals. Hence, it has many applications in agricultural, nonagricultural, and public health purposes[27]. Malathion's targeted pests include ants, aphids, bugs, borer, fruitworm, caterpillars, cattle lice, cockroaches, fleas, flies, grasshoppers, mosquitoes (adult, larvae), spiders, ticks, etc[26]. Besides the genotoxic and neurotoxic effects on insects and plant; pesticides are also found to alter the various stages of cell cycle especially mitotic behavior and S-phase of the cell cycle[29]. Sinha *et al*[28]., studied the cytotoxic effect of Citrinin on relative division rate of *Allium cepa* root cells. He found a marked decrease in the mitotic index with a gradual increase in the concentration of toxin[28].

The present study was sought to evaluate the cytotoxic effect of Malathion in *Allium cepa*. This plant assay was selected because of its cost effectiveness and reliability than other methods for evaluation of chromosomal aberration and for easy access of toxicity at different concentrations.

MATERIALS AND METHODS

Test Chemicals

1. Aceto Alcohol (1:3) Fixative
2. 1% Acetocarmine
3. 2% Acetocarmine
4. Different concentrations of Malathion in percentage (i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9) %.

Sample

Infection free young onion bulbs were collected from the orchard of Department of Life-Sciences, Dibrugarh University, for the experimental purpose.

Treatment

Onion bulbs were kept in distilled water after cutting the lowermost base portion of the bulb and allowed to develop roots at r.t. conditions (25°C). Rooting was carried out in conical flasks. After the development of roots (0.5 mm long) the onion bulbs were kept in different concentration of Malathion. Ten different concentrations [0.1% - 0.9% named as T1 (Treatment-1) - T9 (Treatment-9)] of Malathion pesticide were prepared with d.w. as diluents and as well as the control. Five onion bulbs were planted per concentration with each bulb being placed on 100 mL beaker filled with different concentrations of the pesticide for 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively in each concentration for the recovery period. After the desired time, the roots of each onion bulb of each concentration were collected and fixed in Acetic acid: Ethanol (1:3) fixative and kept for 24 hrs. After 24 hrs, the root tips were taken out and kept in 70% Ethanol and stored at 4°C till hydrolysis.

Hydrolysis

The root tips were hydrolyzed in 1 N HCl and 2% Acetocarmine (1:9) and warmed gently for 5 min without boiling.

Slide preparation

Slides were prepared by Acetocarmine squashing technique[25].

Flowchart representation of the Acetocarmine Squashing Technique

A healthy hydrolyzed root tip was taken on a clean grease free slide. The tip portion of the root was cut and the other parts were removed.

↓

One drop of 1% Acetocarmine stain was added to the slide.

↓

Then, the root tip was covered with a round coverslip and warmed gently.

↓

The slide with the coverslip was placed in between a folded blotting paper.

↓

The root tip was squashed by applying hard and uniform pressure.

↓

Excess stain was blotted off and edge of cover slip was sealed with wax

↓

Now, the prepared slide was observed under a compound microscope.

Mitotic Index (MI) analysis:

The purpose of the mitotic index is to measure cellular proliferation[23].

Mitotic index (MI) was calculated using the following equation:

$$\boldsymbol{Mitotic\ Index\ (MI)} = \frac{\boldsymbol{(P+M+A+T)}}{\boldsymbol{N}} \times \mathbf{100}\%$$

Where, (P+M+A+T) = Summation of all the dividing cells in various phases as Prophase (P), Metaphase (M), Anaphase (A) and Telophase (T).

N = Total number of cells

Statistical Analysis

For the determination of the dividing cell, SD value (Standard Deviation) was calculated.

Formula of the SD value is as follows-

$$\boldsymbol{Standard\ Deviation\ (\sigma)} = \sqrt{\frac{\sum(\boldsymbol{X} - \bar{\boldsymbol{X}})}{\boldsymbol{n}}}$$

where,

X = Total number of cells

$\bar{X}$ = Number of dividing cells

n = Number of observations

RESULTS

The prepared slides of each recovery period of each concentration was observed under the compound microscope using 10X and 40X magnification. Five different microscopic fields were taken randomly to observe the dividing cells. Thus, from each microscopic field, a total number of cells and number of dividing cells were recorded and percentage of dividing cells was calculated. Mitotic

divisional stages were also recorded properly. Separation and accumulation of chromosomes in the center of the cell were recorded and tabulated accordingly (Table 1 and Fig. 1). It was observed that the first mitotic depression occurred within 48 hrs at 0.1% conc. at the rate of ~53% and MI% gradually decreased till ≈ 40% by the end of 96 hrs. But interestingly, when the conc. was increased to 0.2%, although initially till 48 hrs MI% kept on decreasing showing mitotic depression of the root cells, but suddenly there was abrupt increase in MI% on reaching 72 hrs (41.9%) which was almost at the rate of 147 factor. But on further time elapse, *i.e*. till 96 hrs, again there was decrease in MI% suggesting a mitotic depression after the abrupt increase and it was comparatively more than the 48 hrs reading. Now, when the root cells were kept at 0.3% conc. of Malathion, it was observed that within 24 hrs as compared to earlier conc. exposure (MI% at 0.1% = 83.9%; MI% @ 0.2% = 69%); it got reduced by a huge margin ~307 times but again interestingly it again increased when kept at 0.4% (24 hrs) by ~137 times. This anomaly of unevenness was observed commonly on comparing 0.5% to 0.9% 24 hrs data. Thus from this type of anomaly it can be suggested that as the root cells were in various mitotic phases and due to the cellular elongation of actively dividing meristems, the inhibitory effect of Malathion got skewed proportions.

A stimulating observation was noted when the root cells were exposed to 0.4% conc. as the MI% after 24 hrs was 37.5% which kept on increasing above 50% (MI% @ 48 hrs = 50%; MI% @ 72 hrs = 55.3%; MI% @ 96 hrs = 57.1%). Surprisingly, during overnight conditions, chromosome separation was observed from prophase and metaphase but after 72 hrs even during anaphase chromosome separation was observed. So, the possible reason which can be laid for this observation is that may be during 0.4% Malathion exposure, the cells as they were dividing, they also started accumulation the chemical compound within the cells while cell elongation took place. From the observed data, it can be clearly inferred that @0.4% conc. after 48 hrs, there was highest mitotic division which kept on increasing even after reaching 96 hrs. So, this can be termed as the accumulatory phase or threshold accumulation capacity of the cells. This can be again supported by the fact that in all other experimental conditions, none of the varied concentration at any time interval had shown highest mitotic division of the meristems of the root cells under the Malathion exposure. When the conc. was kept on increasing from 0.6% to 0.9%, a general trend was observed where the root cells had shown less mitotic division on comparison to 0.1% to 0.4% at all-time intervals. This showed that there is a concentration dependent cytotoxic inhibitory effect of the Malathion on the root cell mersitems. The least mitotic activity was observed @0.8% conc. at 72 hrs (MI% = 11.1%) and during 0.9% conc. @ 24 hrs (MI% = 11%). The negative control had MI of 73.5%.

The separation of chromosomes during metaphase, early and late anaphase was only observed @ 0.1% conc. @ 24 hrs; whereas the chromosomes got separated during prophase, metaphase and anaphase at 0.3% conc. @ 24 hrs; 0.4% @ 72 hrs and @ 96 hrs. Chromosomes got separated during prophase and metaphase only at 0.4% conc. @ 24 hrs. Similarly, chromosome shrinkage of metaphase and anaphase was observed at 0.7% @ 48 hrs and 96 hrs but chromosome shrinkage during metaphase stage was observed at 0.8% @ 72 hrs and @ 96 hrs. Lastly, chromosome aggregates at the centre was formed at prophase and metaphase.

Table 1. Percentage of Mitotic cell division in Onion root tip after treatment of Malathion

Treatment	24 Hours				48 Hours				72 Hours				96 Hours			
	Total no. of cells	Dividing cells	Mitotic Index	Standard Deviation	Total no. of cells	Dividing cells	Mitotic Index	Standard Deviation	Total no. of cells	Dividing cells	Mitotic Index	Standard Deviation	Total no. of cells	Dividing cells	Mitotic Index	Standard Deviation
Control	98	72	73.5%	± 2.28												
Character : Prophase, metaphase, anaphase																
0.1%	93	78	83.9%	±1.73	54	24	44.4%	± 2.44	59	25	42.3%	± 2.60	99	40	40.4%	±3.43
Character	Metaphase, Early and Late Anaphase, Chromosome separates				Prophase, Metaphase, Anaphase, Telophase				Metaphase, Anaphase, Telophase				Prophase, Metaphase, Anaphase			
0.2%	100	69	69%	± 2.48	74	21	28.4%	±3.25	93	39	41.9%	±3.29	118	39	33.1%	±3.97
Character	Prophase, Metaphase, Anaphase				Prophase, Metaphase				Prophase, Metaphase, Anaphase				Prophase, Metaphase, Anaphase			
0.3%	55	15	27.3%	± 2.81	57	10	17.6%	± 3.06	67	19	28.4	±3.09	79	16	20.3%	±3.54
Character	Prophase, Metaphase, Anaphase, Chromosome separates				Prophase, Metaphase				Prophase, Metaphase, Anaphase				Prophase, Metaphase			
0.4%	64	24	37.5%	± 2.82	72	36	50%	± 2.68	76	42	55.3%	±2.60	98	56	57.1%	±2.89
Character	Prophase, Metaphase, Chromosome separates				Prophase, Metaphase				Prophase, Metaphase, Anaphase Chromosome separates				Prophase, Metaphase, Anaphase Chromosome separates			
0.5%	71	20	28.7%	±3.19	75	16	21.3%	± 3.43	86	22	25.6%	±3.57	77	15	19.5%	±3.52
Character	Prophase, Metaphase				Prophase, Metaphase				Prophase, Metaphase				Prophase, Metaphase			
0.6%	94	29	30.9%	±3.00	78	10	12.8%	± 3.68	67	16	23.9%	±3.19	83	13	15.7%	±3.74
Character	Prophase, Metaphase				Prophase, Metaphase				Prophase, Metaphase				Prophase, Metaphase			
0.7%	89	15	16.9%	±3.84	88	13	14.8%	±3.87	107	15	17.8%	±4.28	110	17	15.5%	±4.31
Character	Prophase, Metaphase Anaphase				Metaphase Anaphase Chromosome shrink				Metaphase Anaphase				Metaphase Anaphase Chromosome shrink			
0.8%	103	14	13.6%	± 4.21	103	20	19.4%	±4.07	108	12	11.1%	± 4.38	110	17	15.5%	±4.31
Character	Metaphase Anaphase				Metaphase Anaphase				Metaphase Chromosome shrink				Chromosome shrink, Metaphase			
0.9%	100	11	11%	± 4.21	106	15	14.2%	±4.27	104	15	14.4%	±4.22	95	15	15.8%	±4.00
Character	Prophase, Metaphase, Late Anaphase				Prophase, Metaphase				Prophase, Metaphase,				Prophase, Metaphase, Chromosomes aggregate at the center			

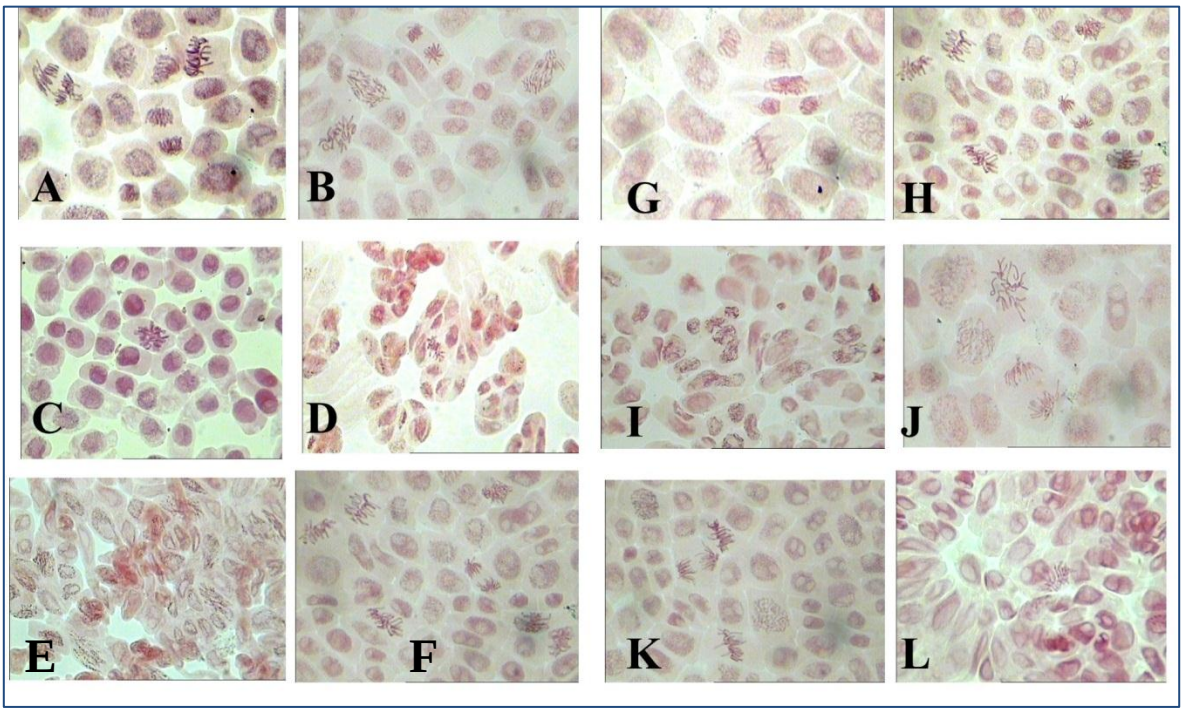

Fig. 1: Effects of Malathion on Mitotic division as observed under microscope (Randomly selected). A = 0.1 %, 24 hours; B = 0.2% 24 hours C = 0.5%, 24 hours; D = 0.6% 24 hours; E = 0.8%, 24 hours; F = 0.9%, 24 hours; G =0.5% 48 hours; H = 0.6%, 48 hours; I = 0.7%, 48 hours; J = 0.3%, 72 hours; K = 0.4%, 96 hours; L = Control

DISCUSSION

Cellular abrasion, cytotoxicity and genotoxicity are the new orders of physioanalytic paradigm observed in dividing plant cells to monitor the toxicological effects of chemical based biocides. Here, in our experiment we have checked and analyzed the toxicological effects of Malathion on *Allium cepa* plant model. Malathion is an OP herbicide or pesticide which is generally regarded as neurotoxic as it irreversibly inactivates acetylcholinesterase (AChE) enzyme rendering neuro associated disorders eventually leading to fatality. Plants are always regarded as good and safe study models for the assessment of various biocides used in agriculture, domestic and forest like environments and their immediate settings. Plants play a crucial role in every biogeochemical cycle as they are both direct and indirect co-associates, in scavenging these used biocides along with the soil micro-flora. These agrotoxic compounds applied to ward off pests gets accumulated in the plant systems. And this property can be used to monitor the cytotoxic effects of the biocides. For the first time, we have reported the potential cytotoxic effect or inhibitory effect on the dividing meristems of *Allium cepa* by Malathion

Evolutionally advanced plants like *Allium cepa*, *Tradescantia* sp., *Crepis capillaris*, *Glycine max*, *Vicia faba*, *Pisum sativum*, *Arabidopsis thaliana*, *Tipula paludosa*, *Zea mays* etc. are the best potential genetic plant systems commonly used for mutagenic or cyto-toxicology experiments as they have large monocentric chromosomes in reduced numbers[21]. Among all of these plant systems, *Allium cepa* assay has been the most effective and extensively used system due to its high predisposition for the determination of cytotoxic and genotoxic impingements of herbicides or biocides[5]. It has also been reported that the *A. cepa* system has shown precisely accurate and good correlation with comparison to other prokaryotic and eukaryotic test systems[21].

Through our experiment, it was observed that there was a gradual increase in mito-depression in the root cells treated with increased Malathion concentration. This increase was very significantly observed in T1, T2, T3, T5, and T6. Interestingly, the T4 concentration showed slightly increased dividing percentage in the sample. The maximum mitotic division was observed in T1 (24 hrs followed by 96 hrs and 72 hrs respectively). Within 24 hrs recovery period, the treatment T1 (0.1%) and T2 (0.2%) had the highest rate of mitotic division. The mitotic depression was subsequently higher in T4 (0.4%) at 24 hrs and 96 hrs respectively. This result can lead to the assumption that there might have been a possible mitotoxic effect on the nascent growing cells.

The possible explanation of the mitotoxicity effect is that the insecticide Malathion being an organophosphate compound and alkalyting agent might have interfered with the normal cell division and the agent might also be involved in the stimulating lyases. In contrary an interesting observation was made from the results is that a minute concentration of Malathion (T1 - 0.1%: MI – 83.9%) can increase the cellular proliferation when compared to mitotic index of control root tip cells (MI – 73.5%). But the MI gradually drops with increasing Malathion concentration as a result of increased mitotic depression. In similar experiments, it was observed that through Allium root growth test, Anilofos (which is also an OP and used in domestic and agricultural purposes) was regarded as cytotoxic as it had caused growth inhibition in the root cells of *A. cepa*[21]. And through many correlated assays it was concluded that this type of inhibition was due to the cellular differentiation occurred during the cell elongation phenomena or apical meristematic activity or the inhibition of protein synthesis[11,24,30].

Mitotic Index is a very dependable argument which has been used commonly for estimation of the frequency of the cellular division[10]. To screen the cytotoxic agents, inhibition of mitotic activities is an important

parameter[16]. In our study we have found that there are two types of responses of MI on the exposure to various conc. of Malathion at different time intervals. The MI% values in some cases were higher (0.1% @ 24 hrs) than the control (negative) which might be due to the result of the induction of cell division. Whereas, in majority of the cases, the MI% values were lower than the control (negative) which again might be due to the growth and development (accumulatory phase) of the cells which were affected by Malathion exposure[12]. From our data, we can state that there was a concentration-dependent inhibition of MI which can be rendered as the possible cytotoxic potential of Malathion in *A. cepa*.

The rate of mito-depression was highest (89%) in the sample treated with T9 (0.9%) with a mitotic index of 11% after 24 hr recovery period followed by T8 (0.8%) 72 hrs and 96 hrs. The experiment showed a significant 50% mito-depression in T4 (0.4%) treatment at 48 hrs. During this period of the experiment, different stages of mitotic cell division *viz.* - Prophase, Metaphase, Anaphase, and Telophase were recorded (Table 1). Besides these phases, the separation of chromosomes in metaphase stage of 0.1% conc. (24 hr), 0.2% conc. (72 hr) and shrinkage of chromosomes in 0.8% conc. (96 hr) and 0.9% conc. (72 hr, 96 hr) had also been recorded. The experiment was marked by the dominant metaphase stage followed by anaphase, telophase stage and few cells with prophase stage. A series of mito-toxicity was observed under the influence of Malathion. The abnormal mitotic phases include disturbed metaphase and anaphase and shrinkage of anaphase chromosomes. In some extreme cases (0.1%, 0.2%, 0.5% treatments) the chromosomes separate or loosened, clearly avoiding the pair formation and in some instances, spindle formation was inhibited (Fig. 1).

The Malathion cytotoxicity can be explicated by its chemical structure. It is believed that OPs do not have direct inhibitory effects on AChE but rather the thioesters of these compounds (P=S) shows or causes inhibitory effects only after conversion to its oxo (P=O) form. Hence, it can be inferred that thioester bond containing chemical compounds or biocides must get converted to their oxo forms for activating the inhibitory effects[17,18]. Therefore, in our view, this compound may be cytotoxic due to its chemical structure and biological activity in the cell. And it is known that any pesticides when penetrates the cellular membrane of the cell they have the general tendency to accumulate and render the cell toxic[4]. This phenomena leads to various affects in mitotic phases which was proved through many workers[2,15,21,22].

In future perspective, it will be really very interesting to examine the types of metaphase-anaphase-telophase aberrations occurred and also what are the total mean percentages of other anomalies caused by Malathion on *A. cepa*. As we have seen, that there was chromosome shrinkage during the metaphase-anaphase so it would be very inquisitive to understand that which type of chromosome aberration caused it. It can be due to disturbed anaphase-telophase, chromosome laggards, stickiness, anaphase bridges or c-metaphase, disturbed nucleus, polyploidy, or due to binuclear cells.

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