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EFFECTS OF INSECTICIDE 0.2% CHLORPYRIFOS 50% E.C., 30% *BALANITES AEGYPTIACA* L. AND 30% *SAPINDUS MUKOROSSI* GAERTN. FRUIT EXTRACTS ON MITOTIC INDEX AND MITOTIC INHIBITION IN ONION (*ALLIUM CEPA* L.) ROOT TIP MERISTEM CELLS

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ABSTRACT
Agrochemicals are extensively used by the farmers for controlling the insect pests. Amongst them organophosphates (OP) and organochlorines (OC) are used regularly. Chlorpyrifos (Agrochemical), aqueous fruit extracts of *Balanites aegyptiaca* L. and *Sapindus mukorossi* Gaertn., were used separately to assess their cytological effects on onion root tip cells. The results revealed that, mitotic index decreased proportionally with increasing Chlorpyrifos treatment period. 30% *B. aegyptiaca* L. fruit extract had stimulatory effect at low treatment periods while its high treatment time had inhibitory effect on dividing cells. 30% *S. mukorossi* Gaertn. fruit extract had stimulatory effect at high treatment time while its low to intermediate treatment time had inhibitory effect on dividing cells. From the study, it can be concluded that, instead of agrochemicals, farmers can use bio-pesticides for controlling the pest.

KEYWORDS: Agrochemicals, Chlorpyrifos , *Balanites*, *Sapindus*, Biopesticides.

INTRODUCTION

At the present time agrochemicals like insecticides, weedicides & fungicides are used by farmers on large scale to control pests, weeds and diseases. Every life is been exposed to these agrochemicals and they have been proved to cause certain chromosomal abnormalities in the crop plants. Molecules like Endosulfan, Malathion, Chlorpyrifos, Methyl demeton, Dichlorvos, Phorate, Dimethoate and Metasystox are found to be positive for controlling insect pest. Unfortunately, research has proved that the use of such pesticides is unfavourable to the soil micro flora. Such chemicals are unsafe to the nature and responsible for air, water and soil pollution (WHO 2010, Wabale *et al.*, 2012). They are also found to have certain cytological abnormalities. Onion is one of the important crops, grown for food as well as its medicinal properties. It is the second most crops which is grown next to tomato and is consumed across the world (FAO, 2012). An attempt was done to test the mitotic index and mitotic inhibitions due to agrochemical Chlorpyrifos and biopesticides prepared from fruits of *Balanites aegyptiaca* L. and *Sapindus mukorossi* Gaertn. on onion root tip cells, as the test plant is easy to handle and easily available. It yields fresh roots and provides meristem cells for study every two or three days.

MATERIALS & METHODS

Matured dry bulbs of onion (*Allium cepa* L.), fruits of *Balanites aegyptiaca* L. and *Sapindus mukorossi* Gaertn. were used for the study. 1% Aceto-carmine reagent was used for staining the cells. Following procedure was tagged along during the investigations.

A) Preparation of 0.2% Chlorpyrifos 50% E.C.: 2 ml of Chlorpyrifos E.C. was mixed with 98 ml water. Final volume was made to 1000 ml to obtain 0.2% Chlorpyrifos 50% E.C.

B) Preparation of aqueous extracts from the fruits of *Balanites aegyptiaca* L. and *Sapindus mukorossi* Gaertn.

Fresh fruits were collected, cleaned and dried. The fruits were powdered and about 250gm of powder from each fruit was used for extract preparation. 500ml of cow urine was added to the powder separately followed by 1200ml of water. The mixture was transferred into 2 litre capacity plastic container and kept for about 5 days. After then, the mixture was transferred into the vessel and boiled to reduce it to half the volume. Further it was filtered through muslin cloth and the filtrate was collected separately for both fruit extracts. From these stock solutions, various concentrations were prepared.

C) Treating of onion roots tips with 0.2 % Chlorpyrifos as well as 30% fruit extracts of *Balanites aegyptiaca* L. *and Sapindus mukorossi* Gaertn. for observing their cytological effects:

Healthy and uniformed sized bulbs were placed on the 50 ml couple jar filled with distilled water at room temperature for 48 hours. As the roots from the sprouted bulbs reached the length of about 0.5cm-1.5cm, they were transferred to beakers containing 0.2 % Chlorpyrifos 50% E.C, 30 % *Balanites aegyptiaca* L. *and Sapindus mukorossi* Gaertn. extracts. They were treated in the insecticide and fruit extracts separately for 1hr, 2hr, 3hr, 4hr and 5hr respectively at room temperature (Fig.1). Bulb roots in distilled water were treated as control. After the

treatment of chemical insecticide and biopesticides, the onion bulb roots were thoroughly washed under running tap water and immediately transferred to the jars containing distilled water (Shaikh S. *et al*., 2012).

Onion bulb treated with tap water

Onion bulbs treated with 0.2% Chlorpyrifos 50% E.C. insecticide

Onion bulb treated with 30% *Balanites aegyptiaca* L. fruit extract.

Onion bulb treated with 30% *Sapindus mukorossi* Gaertn. fruit extract.

FIGURE 1- Treating of onion roots tips with 0.2 % Chlorpyrifos as well as 30% fruit extracts of *Balanites aegyptiaca* L. *and Sapindus mukorossi* Gaertn. for observing their cytological effects

D) Study of cell division with special reference to Mitotic index and Mitotic inhibition

Roots from treated onion bulbs were cut out and root tips were incise and added to distilled water separately. The root tips were preserved in carnoy's fixative (acetic acid: alcohol 3:1) for ten minutes. Preserved root tips were transferred to 70% ethanol and then after 10 minutes they were washed with distilled water.

Further, the root tips were kept in 1N HCl and warmed on low flame of the spirit lamp for near about 2 minutes with alternating cooling. The treatment of HCl (hydrolysis) dissolves the middle lamella and cells get separated and it also allows better penetration of stain. After then, the root tips were transferred to watch glass containing 45% glacial acetic acid. Root tips of 1cm size were taken on clean glass slides with 1 drop of 1% acetocarmine and 1mm tip containing meristem was excised with the help of blade. Further the root tip was smash with the help of pin head and remaining stain was removed. Procedure was repeated for 4-5 times, cover slip was placed with the help of needle without any air bubbles. Care was taken to ensure proper spreading of cells by applying pressure on the cover slip with the help of thumb. Slides were observed under research microscope at 10X, 40X and 100 X magnifications. The stages were photographed with the help of Magnus digital camera, (Asita A.O. and M.M. Mokhobo, 2013).

E) Determination of Mitotic Index (MI) and Mitotic Inhibition:

Mitotic index and mitotic inhibition were calculated from the aceto-carmine stained control and treated onion root tip meristematic cells separately using the following formulas. (Ali A.A., *et al.*, 2015).

$$\textbf{1) Mitotic Index (MI)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells (dividing and non-dividing)}} \times 100$$

$$\textbf{2) Mitotic Inhibition} = \frac{\text{Mitotic index of control- Mitotic index of treated}}{\text{Mitotic index of control}} \times 100$$

RESULTS & DISCUSSION

A) Mitotic index

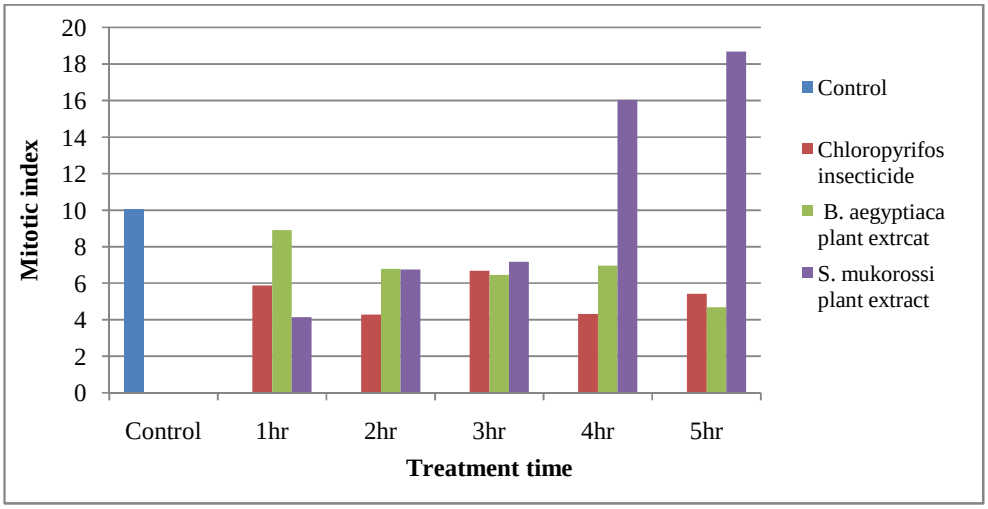
Cytological effect of chemical insecticide as well as fruit extract on exposed roots revealed a dose- dependent decrease in the number of dividing cells with all of the values obtained for treated root cells lower than that of control. The mitotic index (MI) values presented in table showed surprising results. It was noticed that the mitotic index of insecticide treated cells was less than that of treated cell with fruit extracts (Table-1 and Graph-1). The mitotic index value obtained for insecticide and biopesticides was dependent on treatment time.

B) Mitotic inhibition:

Mitotic inhibition was calculated in insecticide as well as fruit extract treated with onion root meristem. It was used to determine the inhibition rate of cell division in cell cycle. Mitotic inhibition values are presented in observation Table-2. From obtained results, it was noticed that in both insecticide and fruit extracts treated with onion root meristem, mitotic inhibition was observed. The mitotic inhibition was observed in different treatment time (1hr, 2hr, 3hr, 4hr and 5hr) in insecticide and both plant extract.

TABLE 1: Effect of 0.2% Chlorpyrifos 50% E.C., 30% *B. aegyptiaca* L. and 30% *S. mukorossi* Gaertn. fruit extracts on Mitotic index in *Allium cepa* L. root tip cells

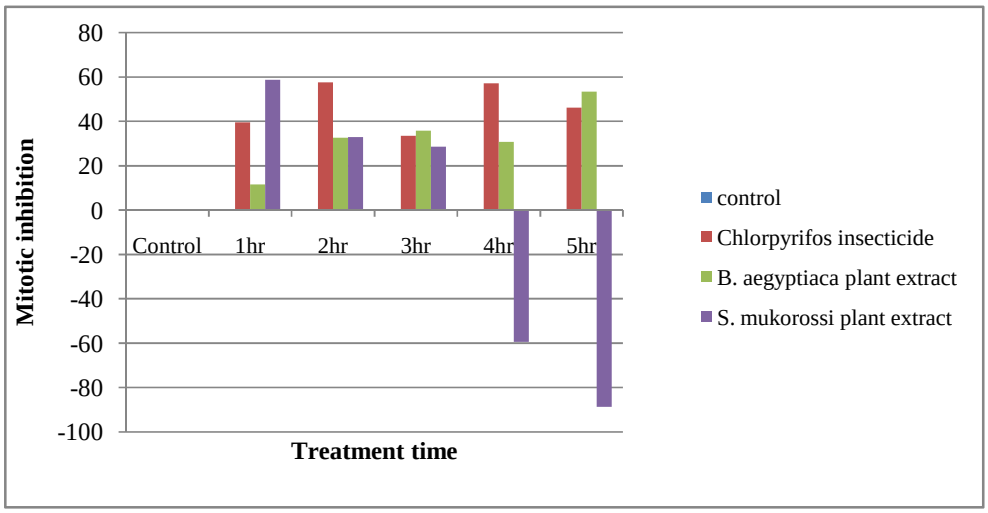
Concentration	Mitotic index (%)
Control	10.06
Insecticide(0.2% chlorpyrifos 50% E.C.)	
1hr	5.88
2hr	4.28
3hr	6.69
4hr	4.32
5hr	5.42
Fruit extract (30% *Balanites aegyptiaca* L.)	
1hr	8.90
2hr	6.79
3hr	6.46
4hr	6.97
5hr	4.69
Fruit extract (30% *Sapindus mukorossi* Gaertn.)	
1hr	4.15
2hr	6.75
3hr	7.18
4hr	16.03
5hr	18.68



GRAPH 1: Effect of 0.2%Chlorpyrifos 50% E.C., 30% *B. aegyptiaca* L. and 30% *S. mukorossi* Gaertn. fruit extracts on mitotic index in *Allium cepa* L. root tip cells.

TABLE 2: Mitotic inhibition caused due to effect of 0.2% Chlorpyrifos 50% E.C., 30% *Balanites aegyptiaca* L. and 30% *Sapindus mukorossi* Gaertn. fruit extracts in root tip cells of *Allium cepa* L.

Concentration	Mitotic inhibition (%)
Control	00
Insecticide (0.2% Chlorpyrifos 50% E.C.)	
1hr	39.43
2hr	57.45
3hr	33.49
4hr	57.05
5hr	46.12
Fruit extract (30% *Balanites aegyptiaca* L.)	
1hr	11.53
2hr	32.50
3hr	35.78
4hr	30.71
5hr	53.37
Fruit extract (30% *Sapindus mukorossi* Gaertn.)	
1hr	58.74
2hr	32.90
3hr	28.62
4hr	-59.34
5hr	-88.68



GRAPH 2: Mitotic inhibition caused due to effect of 0.2% Chlorpyrifos 50% E.C., 30%*Balanites aegyptiaca* L. and 30% *Sapindus mukorossi* Gaertn. fruit extracts in root tip cells of *Allium cepa* L.

DISCUSSION

Verma A., 2011 reported that when onion root tip cells were exposed to the *Aloe vera* extract, a highly significant increase in MI was observed, resulting from an increase in the number of cells in prophase. No increase in chromosomal abnormalities was found, but a significant increase in frequency of total abnormality resulted from marked disturbance in cytokinesis. In present investigation onion root tip treated with 30% *B. aegyptiaca* L. and 30% *S. mukorossi* Gaertn. fruit extracts showed increased mitotic index. Rao *et al*., 1988, studied cytological effects of herbicides and insecticides on *Allium cepa* root tip meristem. Results from the research showed that the mitotic indices gradually decreased with increase in time and concentration gradient. In the present investigation chemical insecticide as well as fruit extract on exposed roots revealed a dose- dependent decrease in the number of dividing cells with all of the values obtained for treated root cells lower than that of control. It was noticed that the mitotic index of insecticide treated cells was less than that of treated cell with fruit extracts The mitotic index value obtained for insecticide and biopesticides was dependent on treatment time.

CONCLUSION

In present investigation the mitotic index and percent mitotic inhibition in *Allium cepa* L. root tip meristem cells was carried out by treating them with 0.2% Chlorpyrifos 50% E.C. and fruit extracts of 30% *Balanites aegyptiaca* L. and 30% *Sapindus mukorossi* Gaertn. The mitotic index was minimum in 0.2% Chlorpyrifos 50% E.C. The mitotic inhibition increased with increase in the concentration of 0.2% Chlorpyrifos 50% E.C. The result showed that mitotic index decreased proportionally with increasing insecticide treatment period. The 30% *B. aegyptiaca* L. fruit extract had stimulatory effect at low treatment period while its high treatment time had inhibitory effect on dividing cells. Hence, 30% *B. aegyptiaca* L. fruit extract treated root tip cells showed decreased mitotic index with increase in treatment period. But in 30% *S. mukorossi* Gaertn. fruit extract had stimulatory effect at high treatment time while its low to intermediate treatment time had inhibitory effect on dividing cells. Hence the 30% *B. aegyptiaca* L. and 30% *S. mukorossi* Gaertn. fruit extracts, are not highly toxic for the plant growth. From the result it can be concluded that the synthetic insecticides leads to various cytological changes in the plants. The plant extract showed fewer changes as compared to synthetic chemical. So from presence investigation it can be suggested that farmers can go with bio-pesticides instead of synthetic chemical pesticides as it will is easy to prepare, safe, and affordable.

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