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Evaluation of cytotoxicity induced by cypermethrin on onion root tip cells and its mitigation by *Piper nigrum* seeds (black pepper)

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Abstract: *Cypermethrin, a widely used synthetic pyrethroid insecticide is known for its neurotoxic effects on target pests. However, its impact on non-target organisms and plant systems remains a subject of concern. In this study, we investigated the cytotoxiceffect of cypermethrin on onion (Allium cepa) rootsas a model system. Onion root tips were exposed to 20% concentration of cypermethrin for 24 hours, followed by cytotoxicity assessment using mitotic index (MI) and chromosomal aberration (CA) assay.Our results revealed a concentration-dependent decrease in MI, indicating inhibition of cell division and a concomitant increase in chromosomal frequency, suggesting genotoxicity. Additionally, cypermethrin induced oxidative stress, as evidencedby elevated levels of reactive oxygen species (ROS) in onion root cells. These findings underscore the cytotoxic potential of cypermethrin in plant systemsand highlight the need for further research on its environmental impact and regulatory management.*

Key Words: *Cypermethrin, cytotoxicity, MitoticIndex, Onion root tips, Black pepper.*

1. INTRODUCTION:

In the ever-expanding assortment of human existence, the world as a dynamic and complex thread, weaving together an intricate narrative growth of diversities, development and challenges, according to sources, world population has risen at arate of 1.9% per year since 1960. Increasing population brings new challenges towards development. One of these challenges people face indaily lifestyle is the shortage of food production in natural condition. To overcome this problem and forwide production of foods in large scale, many chemical fertilizers, insectides and pesticides came to use. Insects or pests are the biggest threat to crops as theycan consume plant foliage, root and shoot system and soil's fertility. To reduce the effect of insects andto produce healthy foods in good quantity, we add insectides to the soil. The pervasive use of chemicalslike insecticide and pesticides in agriculture has undeniably revolutionized crop production, enhancing yields and safeguarding food supplies.

However, this convenience comes at a cost to the eco-system. Pesticides or insecticides designed to combat pests and disease can adversely affect the soil health, disrupt the balance of microorganisms and potentially leading to long term ecological consequences. But these health effects do not stop the use of insecticide sand pesticides in anyways and in this contrast, synthetic insectides at present time have gained its popularity due to severe known down effect on insects. Point and non-point source pollution is a major or environmental issue in many aquatic ecosystems asboth organic and inorganic toxic chemicals can be added to the aquatic by intentional or unintentional human activities. These toxic chemicals can be absorbed by particulate matter in the water column and deposited in sediments and are subjected to various transformation processes within sediments toact as both a sink and a source of contaminants to the overlying water column and biota (Wang, et al, 2015).

Insecticides are chemicals aimed to actively treatingplant crops to combat insects. Cypermethrin and Malathion are organophosphorus insecticides widely used in agriculture to control insects. WHO classifies cypermethrin as moderately dangerous (Class II). Cypermethrin and Malathion have neurotoxic effects on the peripheral and central

nervous system of mammals and insects (WHO, 1989). Intensive use of malathion polluted food and drinking water can also lead to human mutagenic/ carcinogenic substances while Demirhan et al, detected a significance in farmers exposed to pesticides. Cypermethrin is a synthetic pyrethroid which is derived from pyrethroid, a naturally occurring substance extracted from the chrysanthemum spp. Flower. It has wide usage in agriculture and veterinary, insecticides for the control of many insects like cockroach, mosquitoes, lice, ticks and mites (Shipakar and Karki 2021). Cypermethrin is moderately persistent in soil. Cypermethrin degradesmore rapidly in sandy soil compared to clay soils and in soil with low organic contents, the half-life is 0.5 - 8 weeks (about 2 months). Cypermethrin is more persistent under anaerobic conditions. Cypermethrin is subjected to photodegradation andmicrobial degradation under aerobic degradation.

Cypermethrin binds strongly to soil particles and possesses minimal leaching concern. It hydrolysis slowly under acidic or neutral conditions.

Cypermethrin's concentration decreases rapidly dueto absorption to sediments, particles and plants.

Cypermethrin gaining ground in agriculture, veterinary and in house pest control programmed dueto severe breakdown effects on insects and environmental compatibility over chlorinated hydrocarbons used in insecticides and pesticides but shows less toxicity towards mammals. Thus compounds have high toxic effects on lower aquatic organisms. However, we have sufficient yet controversial data of cytotoxic effect of cypermethrin on mammals as well as microbes.

Plants are still left in this process, even though theyare primary recipients of agricultural compounds.

The cytotoxicity of these compounds has been found to be negative in microbial test systems. Some showvivo chromosomal aberration, micronuclei and chromosomal cells of Allium cepa and it is needed an alternate with less cytotoxicity.

Black pepper (*Piper nigrum*) an Indian native spice has been widely used in human diet for several thousand years. It has several sharp characteristics and stinging qualities attributed to the alkaloid piperine, which also made it the center interest of modern medical researchers. It is historically used not only in human diet but also in traditional medicines and home remedies. The use of black pepper in medicine in ayurveda siddha and unani medicine in India. The 5th century Syria book of medicines prescribes black pepper for illnesses suchas constipation, diarrhoea, earache, gangrene, heart disease, hoarse problem, ingestion, insect bites, liverand toothache. Piperine has been demonstrated in invitro experiments to protect against oxidative damage by quenching free radicals and reactive to oxygen species and inhibiting lipid peroxidation.

Piperine is shown to be against oxidation of human low-density ion-induced lipid peroxidation of human LDH.

The oleoresin of black pepper ranges between 10 and 15% and it contains volatile oil, non-volatile oil,resins, fixed oil, color, sugar etc. Pepper ethanol extracts and volatile oils can preserve food from spoilage (Sharma S, et al 2002). Many researchers are exploring the cytotoxicity pf plants as well as the effects of antioxidants on them.In this study, we aimed to investigate the cytotoxic effect of cypermethrin on onion roots using a combination of cytotoxic assays and oxidative stressmarkers and how black pepper helps to mitigate the cytotoxic effect of cypermethrin because of its medicinal properties.

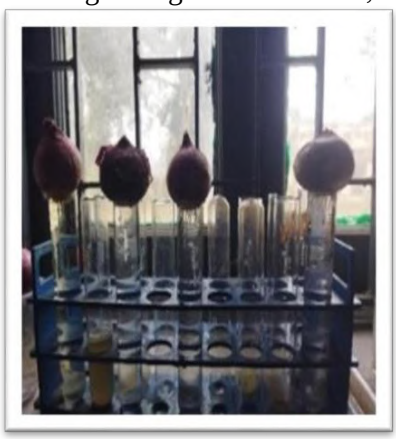
2. MATERIALS AND METHODS:

The cypermethrin solution was prepared using asynthetic insecticide (UPL Ustaad) 10 EC.

The concentration of cypermethrin is taken as 2% which means the solution is made with 2ml (about 0.07 oz) cypermethrin and 98ml (about 3.31 oz) of distilled water(2ml +98ml = 100ml of stock solution). Then a black pepper solution was prepared using 4gm of black pepper powder (crushed using mortal pestle) in 96ml of distilled water. The

final solution of concurrent was also prepared by using both black pepper and cypermethrin with 4ml solution and 2ml solution respectively.

3 bulbs of onions were taken for each solution and the other 3 were taken for control which is a total 12in number. After growing 1 to 2 cm roots, these are grown in prepared solution for 72 hours.



Control was also maintained with no treatment and preserved with 70% alcohol. Acetocarmine stain squash preparation weremade.

Formula used

- $Mitotic\ Index = \frac{Total\ No.of\ Dividing\ cell}{Total\ No.of\ Cell} \times 100$
- $S.\ ERROR\ = \sqrt{\frac{\%\times(100-\%)}{N}}$
- $t\text{-}diff = \sqrt{\frac{\%\ of\ treated - \%\ of\ control}{(S.E.of\ treated)^2 + (S.E\ of\ control)^2}}$

3. RESULTAND DISCUSSION:

Onion bulbs were kept in solution of cypermethrin, Piper nigrum (Black pepper) to study the harmful effect of cypermethrin and therapeutic effect of black pepper when applied to plants at various developmental stages. 4 sets of onions were dividedinto 4 groups, first is the control, second group weretreated with cypermethrin solution, third group is given concurrent treatment with cypermethrin and black pepper mixed, and fourth group was treated with black pepper. After some days, roots were observed morphologically. Results show that roots under cypermethrin were found to be 1.9cm (about 0.75 in), 2.0cm (about 0.79 in), 1.5cm (about 0.59 in), 2.3cm (about 0.91 in), 1.1cm (about 0.43 in), the maximum length found was 2.3cm (about 0.91 in).

Roots under mixture of cypermethrin and blackpepper (concurrent) were found as of length 2.3cm (about 0.91 in), cm (about 0.98 in), 2.7cm (about 1.06 in), 2.9cm(about 1.14 in), comparatively longer than cypermethrin one. Roots under black pepper are if length 3.3cm (about 1.3 in), 4.0cm (about 1.57 in), 4.6cm (about 1.81 in), 4.5cm (about 1.77 in), comparatively longest and the heathiest of all and last roots under control were of length2.8cm (about 1.1 in), 3.0cm (about 1.18 in), 2.9cm (about 1.14 in) , 3.1cm (about 1.22 in.)

Table 1 : toxic effect of cypermethrin and black pepper on root length of onion root tip cells.

Mixture:	**Root Lengths (in cm):**			
Cypermethrin	1.9	1.5	2.0	2.3
Concurrent (C + Bp)	2.3	2.5	2.7	2.9
Black pepper	3.3	4.0	4.5	4.7
Control	2.8	3.0	2.9	3.1

Exposure of onion roots to cypermethrin resulted in notable cytotoxic effects, as evidenced by changes inmitotic index (MI) and frequency of chromosomal aberration (CAs). There were 288 cells which are dividing out of 2013 cells with lots of abnormalities.A concentration dependent decrease in MI was observed, indicating inhibition of cell division following cypermethrin exposure. This inhibition of mitosis suggests that cypermethrin interferes with the normal progression of the cell cycle, potentially disrupting cellular processes. Concomitantly, an increase in the frequency of chromosomal aberrations such as **double metaphase, double anaphase, lagging chromosomes, bridge formation during anaphase, hyper ploidy, bi- nucleated cells, polyploidy** was observed in onion root cells exposed to cypermethrin.

On the other hand, results obtained from the root tipcells exposed to concurrent treatment of cypermethrin and black pepper. Showed that there were 170 cells in dividing stage out of 2300 cells. This result shows that therapeutic treatment of black pepper along with cypermethrin as a potent toxic agent have recovered thr frequency of damaging the cells. Since black pepper is an antioxidant rich harbal spices and thus disrupts the production of free radicals which leads into the production of ROS the antioxidant inside the Black pepper i.e piperine however, combat with the production of free radicals result from toxic particles of cypermethrin and thus prevents the damages to internal milieu of cells.

Table -2 cytotoxic effect of cypermethrin and black pepper on mitotic index and their phases in onion root tip cells

	Totalno ofcells	No of dividing cells	%± SE	T diff.	Phases	No of cells	%±SE
Control	2446	150	6.13±0.48		Prophase Metaphase Anaphase Telophase	72 25 28 23	2.94±0.34 1.02±0.2 1.14±0.25 0.94±0.12
Cypermethrin	2013	288	14.3±0.77*	9.07	Prophase Metaphase Anaphase Telophase	92 37 39 32	4.57±0.47 1.83±0.3 1.94±0.3 1.59±0.28
Black pepper	2300	168	7.3±0.53	1.6	Prophase Metaphase Anaphase Telophase	68 23 32 28	2.95±0.35 1±0.2 1.39±0.24 1.2±0.22
Cypermethrin+Black pepper	2500	170	6.8±0.5	0.97 Or1	Prophase Metaphase Anaphase Telophase	72 28 36 34	2.88±0.33 1.12±0.20 1.44±0.24 0.96±0.19

Here* indicates value at 5% level of significance in comparison to control value.

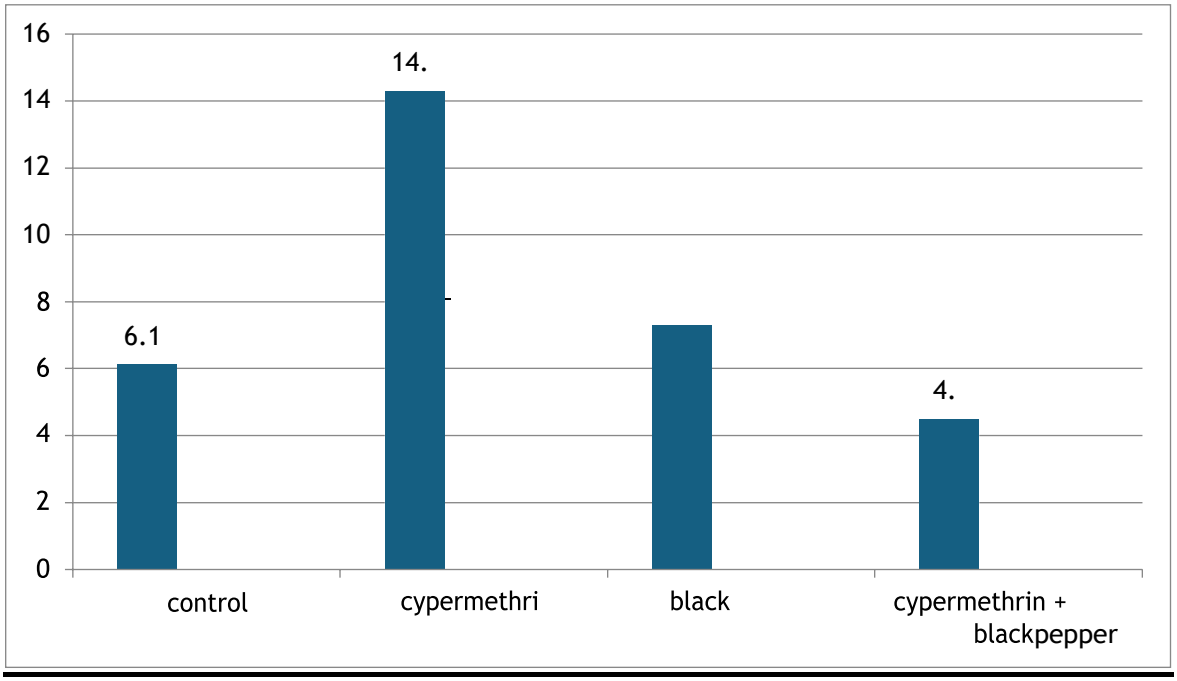


FIG:- Graph showing percentage of cells in division

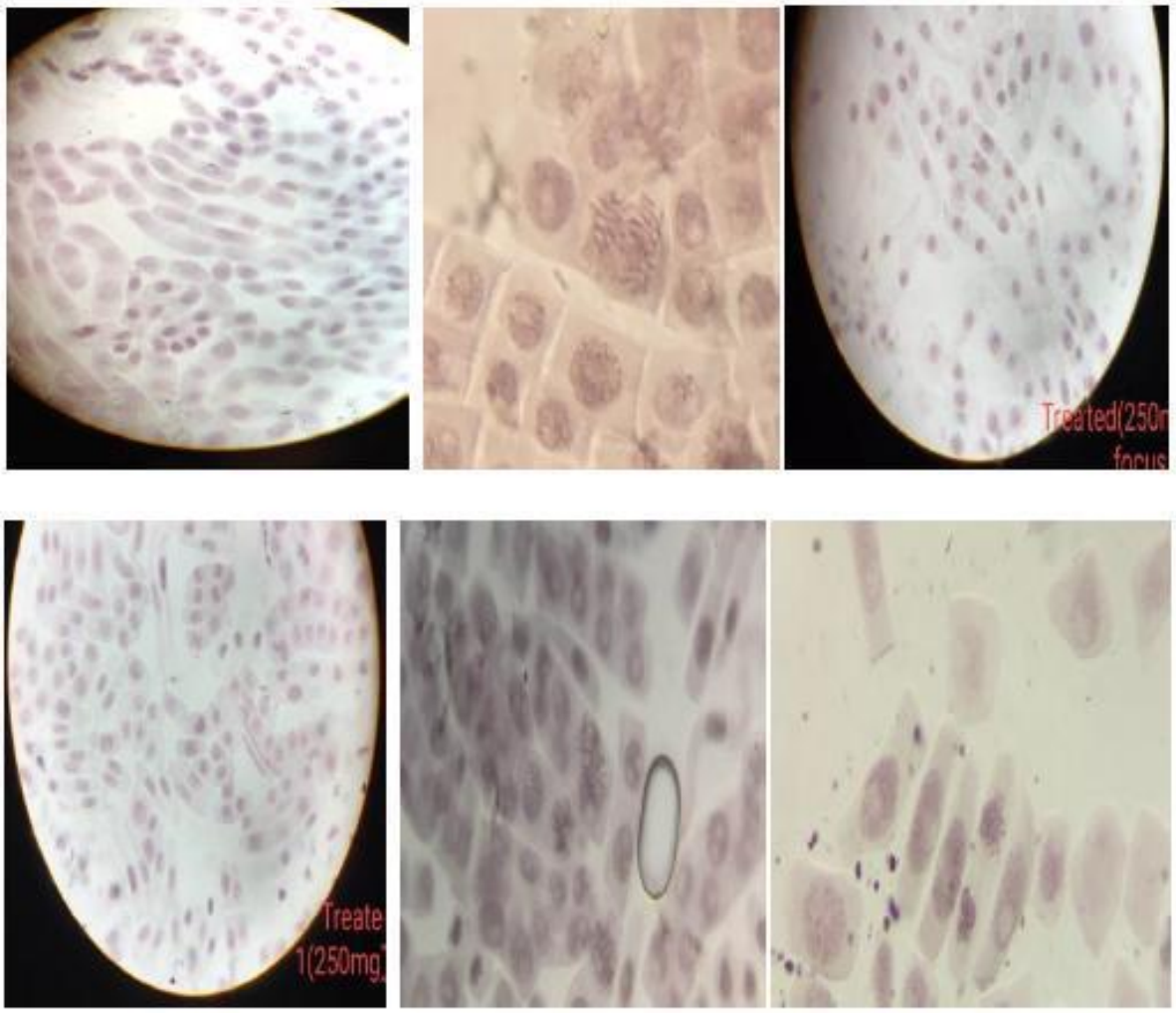


Fig. – Showing the several abnormalities like double anaphase, multipolar spindle, double prophase observed in microscope.

4. CONCLUSION AND SUMMARY:

In this study, we investigated the cytotoxicity effectof cypermethrin on onion root tip cells as a model system for assessing pesticides induced toxicity in plant cells. Cypermethrin is a potent cytotoxic agentand it exerts it's cytotoxic effect by increasing free radicals formation which leads to the production of ROS inside the root tip cells therby it leads into the several chromosomal abnormalities and also suppress the inhibitory effect on the growth of root tips. Our finding reveals that cypermethrin exposure resulted in a concentration dependent decrease in themitotic index (M, indicator of inhibited cells division, and an increase in chromosomal aberration(CAs), suggesting gentoxity. These cytotoxic effects underscore the potential ecological risks posed by cypermethrin in agricultural ecosystem, particularly to non-target organisms such as plants.

Furthermore, our study Elucidated the role of oxidative stress in mediating cypermethrin induced cytotoxicity in onion root tip cells, as evidenced by elevated levels of reactive oxygen species levels of reactive oxygen species (ROS). Oxidative stress is known as disrupt cellular homeostasis, leading to cellular damage and dysfunction. The observed increase in ROS level highlights the need for furtherinvestigation into the mechanism underlying cypermethrin induced oxidative stress and its impacton plant physiology. The cytotoxicity effect of cypermethrin observed in the study have important implications for agricultural practices and environmental management sustainable pest management strategiesthat minimize reliance on chemical pesticides and promote integrated pest management (IPM).

Overall, our research contributes to a better understanding of cytotoxicity mechanism of cypermethrin in plant system and provides valuable insights for assessing the environmental implications of pesticides as well as its mitigation by antioxidant rich black pepper.

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