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Research article

PATTERN OF THE SENSITIVITY OF *ALLIUM CEPA* ROOT MERISTEM CELLS TO ENDOSRI, NUVAN AND KVISTIN PESTICIDES

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ABSTRACT: Cytotoxicity of two insecticides (Endosri & Nuvan) and one fungicide (Kvistin) was evaluated using *Allium cepa* (Onion) root inhibition test. Onion bulbs were exposed to different concentrations of Endosri (2000, 1000, 500, 250, 125, 60, 30, & 15ppm), Nuvan (400, 350, 300, 250, 200, 150, & 100ppm) and Kvistin (3000, 2000, 1000, 750, 500, 250, & 125ppm) for 5 days. Set of bulbs treated with distilled water (DW) was kept as the control. Effects on the growth and number of roots were recorded after the exposure period. EC50 values obtained for Endosri, Nuvan and Kvistin were 60, 200 and 500ppm, respectively. Both Endosri and Nuvan showed highly toxic effect and a significant ($p \leq 0.05$) decrease in both root length and number as compared to control. However, Kvistin was less toxic as compared to Endosri and Nuvan. Our results indicated that even very low concentrations of Endosri and Nuvan are highly toxic to *Allium cepa* root meristematic cells as compared to Kvistin fungicide. This toxicity could also contribute to several afflictions in other organisms after exposure to higher concentrations and a negative impact on the crop production as well.

Key words: Cytotoxicity, *Allium cepa*, Endosri, Nuvan, Kvistin, EC_{50}.

INTRODUCTION

Pesticides are used to control various diseases of crops in India and in other parts of the world. Despite their role in preventing the crops from various diseases, they suffer an untoward result on the biotic factor of the ecosystem, including human beings [1]. Out of 834 registered pesticides by the European Union in 2001 [2], International Agency for Research on Cancer [3] has marked some pesticides as potential or probable mutagens and/or carcinogens.

Endosri is one of the commercially formulated pesticides having Endosulfan (35% EC) as the active ingredient. This is practiced as an insecticide in various crops like Jute, Paddy, Maize, Wheat, Gram, Mustard, Bhindi, Chilly, Cotton to control Jassids, Aphid, Thrips, White Flies, Leaf roller, Bihar hairy caterpillar, Yellow mites, Stem borer, Gall midge, Pink borer termites, Caterpillar, Rice hispa and Termite. Endosulfan belonging to the organochlorine group of pesticides has been widely utilized due to wane in the diligence of other insecticides of the same group such as endrin and DDT [4]. So, being a global pesticide, endosulfan has been in use for about 5 decades and is effective against a liberal bit of insect pests and mites [5-10]. In India this pesticide is used in great measure in various farming practices [11].

Nuvan, another insecticide used in the present study containing Dichlorvos (76% EC) as its active compound, belongs to organophosphate (OP) group, is practiced to handle domestic animals and farm animal and being a commercial insecticide is also practiced to protect homes and crops from insect infestation.

Kvistin is one of the most widely used agricultural systemic fungicides. Its active compound carbendazim (50% WP) is a primary metabolite of the broad-spectrum fungicide benomyl. Carbendazim is also known as Methyl 2-benzimidazolecarbamate (MBC), a systemically active benzimidazole compound that is widely used as an agricultural and horticultural fungicide/pesticide around the world [12]. The mechanism of action for MBC involves the binding to tubulin and subsequent inhibition of microtubular function [13,14].

Various higher plants like *Vicia faba*, *Tradescantia paludosa*, *Pisum sativum*, *Hordeum vulgare*, *Nicotiana tabacum* and *Allium cepa* have been used as model plant systems for general toxicity studies due to the ease of assessing several genetic adversities ranging from a point mutation to chromosomal aberrations [15]. *Allium cepa* is generally used to evaluate toxic potential of pesticides [16-20]. The toxicity tests include determination of EC50 where the growth rate is half compared to control and genotoxicity (amount of chromosomal damage [21].

The state of Jammu and Kashmir (India) is very plentiful in fruit and vegetable crops that contribute a major part to our economy. For getting better crop yields, the local people here use different pesticides. The application of these chemicals to our crop plants is a serious menace to local biota in general and pesticide exposed human population in particular. The present study was undertaken to evaluate the comparative toxic potentials of Endosri, Nuvan and Kvistin by examining root growth, number of roots, root morphology so as to develop the EC50 of these chemicals using root meristem cells of *A. cepa*.

MATERIALS AND METHODS

Organism

Allium cepa (2n=16) onion bulbs, 25-30mm diameter, without any treatment were obtained from a local market (Srinagar, Jammu and Kashmir, India).

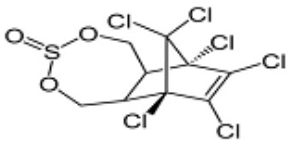
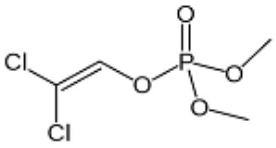
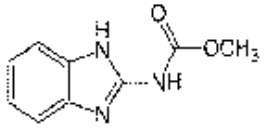
Chemicals

In the present study Endosulfan (6,7,8,9,10-10 hexachloro 1,5,5a,6,9,9a-hexahydro-6-methano- 2,4,3-hexadithioxanthiepin 3-oxide) represented by Endosri (35% EC), Dichlorvos represented by Nuvan (76% EC) and Carbendazim represented by Kvistin (50% WP) were chosen on the basis of their wide use in the local agricultural practices. These pesticides were procured from the registered pesticide dealers. Some chemical properties of these pesticides are provided in Table 1.

Preparation of test concentrations

Pesticides were dissolved in distilled water (dH2O) (as per recommendations to use in the agriculture) in order to prepare the desired ppm (parts per million) concentrations. Initially stock solutions of each pesticide were prepared and other concentrations were obtained by diluting the stock solution. Distilled water was used as control and run parallel at each exposure period and concentration [22].

Table 1: Detailed information of the pesticides used in the present study

Pesticide	Active compound	Chemical group	Molecular formula	Chemical structure of active compound	Usage
Endosri	Endosulfan (35% EC)	Organochlorine	$C_9H_6Cl_6O_3S$		Insecticide
Nuvan	Dichlorvos (76% EC)	Organophosphate	$C_4H_7Cl_2O_4P$		Insecticide
Kvistin	Carbendazim (50% WP)	Benzimidazole	$C_9H_9N_3O_2$		Fungicide

Determination of EC50

EC50 of all the three pesticides against *Allium cepa* (2n□16) was determined according to Fiskesjo [23] and Chauhan and Sundararaman [24]. The healthy and uniform onion bulbs equal in size were chosen from a population of commercial variety. They were not treated with any pesticide or growth inhibitor previously. These were placed over the coplin jars filled with test concentrations of Endosri (2000, 1000, 500, 250, 125, 60, 30, 15 ppm), Nuvan (400, 350, 300, 250, 200, 150 ppm) and Kvistin (3000, 2000, 1000, 500, 250, 125, ppm). For each sample as well as for control (dH2O), five onion bulbs were used. The roots were protected from direct sunlight in order to minimize fluctuations in the rate of cell division as earlier reported by Sharma and Vig [18]. The test concentrations were renewed each day with fresh one throughout the experiment at room temperature (22 ± 2ºC). On the 5th day root length were measured from each treatment (ten roots from each bulb). Taking root lengths of control group as 100%, lengths of experimental groups were plotted against test concentrations and the point showed 50% growth was designated as EC50 concentration. All the experiments were carried out in triplicate.

RESULTS

Table 2 shows the effect of various concentrations of Endosri on mean root length and number of roots after 5 days of treatment duration. It was observed that both the parameters showed a decreasing trend as concentration increased. Control showed the highest average root length and the lowest was observed in 2000ppm of Endosri. The lowest concentration of Endosri (15ppm) showed 85.06% of growth as compared to control (100%). The growth of root length decreased continuously till 500ppm which showed 9.02% of growth compared to control and the next concentration i.e. 1000ppm showed an increase in growth (12.51%). 60ppm concentration produced approximately half of root length of control so this concentration was considered as EC50 (Fig 1-a). In case of root length control exhibited highest average number of roots (105.8) and the lowest were observed in 2000ppm Endosri concentration. However, few concentrations showed a higher number of roots than their successor concentrations like 125ppm which exhibited a lower number of roots than its next concentration i.e. 250ppm. There was a statistically significant difference in the number of roots according to control.

Table 2: Effects of different concentrations of Endosri (35 % EC) on root length and number of roots of *Allium cepa* after 5 days

Treatments	Concentrations (ppm)	Average root length (Cm) ± SD	Growth (%)	Decrease in growth	Average number of roots ± SD*
Control	DW	7.43±0.23^{c}	100	0	105.8±23.63^{c}
Endosri	15	6.32±0.92^{c}	85.06	14.94	80.00±14.93^{bc}
	30	4.70±0.67^{b}	63.26	36.74	65.20±14.46^{ab}
	60	3.62±0.44^{b}	48.72	51.28	56.00±16.09^{ab}
	125	1.46±0.32^{a}	19.65	80.35	42.20±13.79^{a}
	250	1.17±0.43^{a}	15.75	84.25	49.33±18.01^{a}
	500	0.67±0.77^{a}	9.02	90.98	50.00±13.00^{a}
	1000	0.93±0.46^{a}	12.51	87.49	49.33±9.50^{a}
	2000	0.51±0.15^{a}	6.86	93.14	40.66±13.42^{a}

DW: Distilled water; SD: Standard deviation. Means (± SD) followed by different letters are significantly different at the $p \leq 0.05$ level according to Duncans test.

Table 3 summarizes the response shown by the various concentrations of Nuvan. Root length of the test plant was reduced with the increase in concentration. It was observed that 200ppm Nuvan concentration showed 49.53% of root decrease as compared to control and was designated as its EC50 (Fig. 1-b). The 400ppm concentration was highly toxic to the plant roots and only 0.96cm of roots was observed as compared to control which exhibited 7.43cm of roots after 5 days of treatment duration. Average number of roots decreased from 105.8 in case of control to 35.67 in the highest concentration (400ppm) of Nuvan. Root number showed a different trend as compared to root length for varying concentrations of the pesticides.

Kvistin was less toxic as compared to Endosri and Nuvan although the root length and number of roots decreased as the concentration increased. The 500ppm concentration was found to be EC50 as it produced approximately half of root length of control (Table 4, Fig 2-c). The 3000ppm concentration showed lowest root growth and it was found to be 25.17% as compared to control. No significant difference ($p \leq 0.05$) between control, 125 and 250ppm concentrations in case of root length while as, a significant difference was observed in all other concentrations with respect to control. Number of roots decreased as the concentrations increased and lowest number of roots were observed in 3000ppm Kvistin concentration. Here also no significant difference was observed between control and 125ppm concentration.

Table 3: Effects of treatments with different concentrations of Nuvan (76% EC) on root length of *Allium cepa* after 5 days

Treatments	Concentrations (ppm)	Average length (Cm)±SD	Growth (%)	Decrease in growth	Average number of roots ±SD
Control	DW	7.43 ± 0.23^{e}	100	0	105.8 ± 23.62^{d}
Nuvan	100	4.81 ± 0.81^{d}	64.74	35.26	62.00 ± 10.81^{bc}
	150	4.05 ± 0.46^{cd}	54.51	45.49	69.33 ± 3.78^{c}
	200	3.68 ± 0.92^{cd}	49.53	50.47	44.33 ± 11.84^{abc}
	250	3.01 ± 1.2^{bcd}	40.51	59.49	55.67 ± 11.01^{abc}
	300	2.21 ± 0.81^{abc}	29.74	70.26	52.00 ± 4.58^{abc}
	350	1.14 ± 0.24^{ab}	15.34	84.66	43.00 ± 11.35^{abc}
	400	0.96 ± 0.64^{a}	12.92	87.08	35.67 ± 4.16^{a}

DW: Distilled water; SD: Standard deviation. Means (± SD) followed by different letters are significantly different at the $p \leq 0.05$ level according to Duncans test.

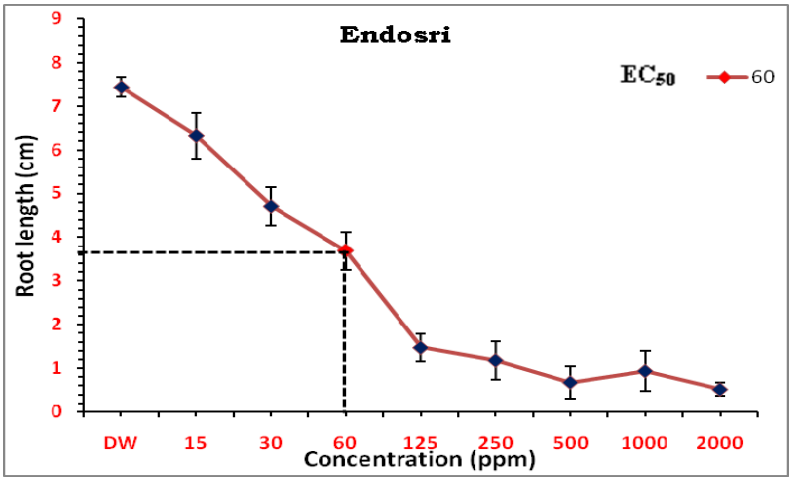
Table 4: Effects of treatments with different concentrations of Kvistin (50 % EC) on root length of *Allium cepa* after 5 days

Treatments	Concentrations (ppm)	Average length (Cm) ± SD	Growth (%)	Decrease in growth	Average number of roots (± SD)
Control	DW	7.43 ± 0.97^{b}	100	0	105.8 ± 23.62^{c}
Kvistin	125	6.60 ± 1.41^{b}	88.83	11.17	96 ± 13.07^{c}
	250	5.78 ± 1.56^{b}	77.79	22.21	75 ± 20.07^{abc}
	500	3.34 ± 1.15^{a}	44.95	55.05	88 ± 25.63^{bc}
	750	2.88 ± 1.16^{a}	38.76	61.24	91 ± 28.35^{bc}
	1000	2.52 ± 1.02^{a}	33.92	66.08	67 ± 17.08^{abc}
	2000	2.28 ± 0.32^{a}	30.69	69.31	54 ± 15.00^{ab}
	3000	1.87 ± 0.77^{a}	25.17	74.83	47 ± 17.08^{a}

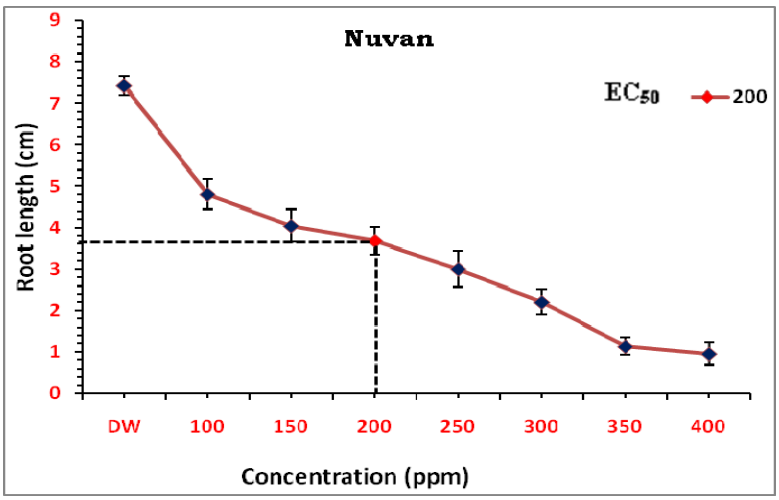
DW: Distilled water; SD: Standard deviation. Means (± SD) followed by different letters are significantly different at the $p \leq 0.05$ level according to Duncans test.

Macroscopic effects induced in *Allium cepa*

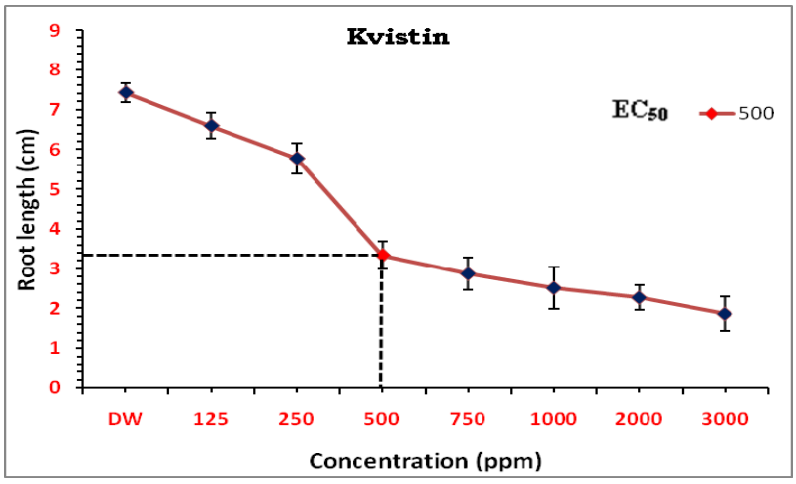
In case of control roots were long, thin and normal growth was observed (Fig. 2-a). The roots of the bulb grown in Endosri and Nuvan showed asymmetrical growth (Fig. 2-b). Here some roots were long while others were very short without uniform growth of roots was observed as in case of control. Higher concentrations of Endosri (500, 1000 and 2000ppm) and Nuvan (300, 350 and 400ppm) showed milky-white and yellowish color of roots (Fig. 2-d). Yellow color of *Allium* roots was also observed in 2000 and 300ppm concentrations of Kvistin. Endosri (1000 and 2000ppm) and Nuvan (350 and 400ppm) induced root swellings or root tumors (Fig. 2-e). Kvistin did not induce any c-tumors at all. Crochet hooks (Fig. 2-d) were observed in Endosri (1000 and 2000ppm), Nuvan (300, 350, 400ppm) and in one concentration of Kvistin (3000ppm). Besides these abnormalities broken root tips (Fig. 2-f) were observed in Nuvan (350 and 400ppm). Kvistin showed less toxicity as compared to Endosri and Nuvan.



(a)



(b)



(c)

Figure 1: Effect of different concentrations of (a) Endosri, (b) Nuvan and (c) Kvistin on *Allium cepa* root length after 5 days treatment duration

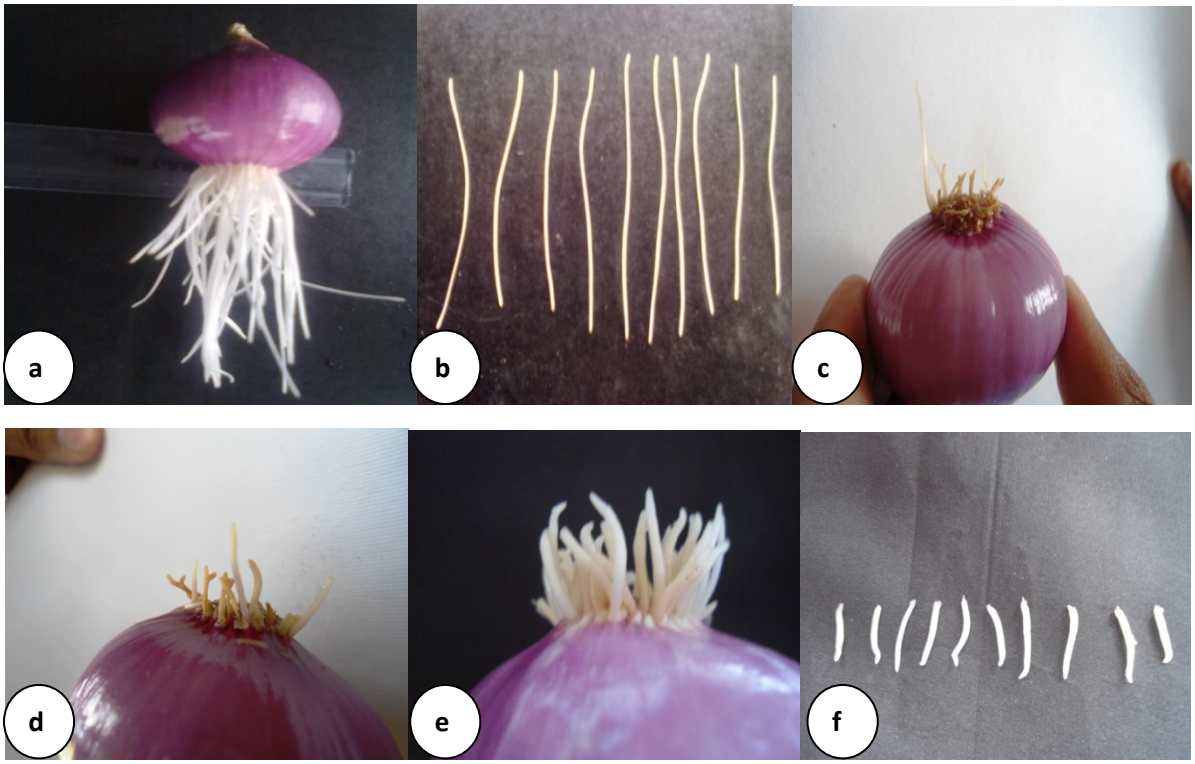


Figure 2: Examples of various macroscopic effects on *Allium* roots treated with various concentrations of pesticides (Endosri, Nuvan, and Kvistin) after five days. (a) Control roots; (b) Control roots spread on a black sheet for counting and measurement of length. (c) Uneven root length and yellow coloured roots in a pesticide treated onion bulb. (d) Pesticide treated bulb showing 'crochet hooks' and yellow coloured roots. (e) C-tumors in a pesticide treated bulb and broken tips. (f) Pesticide treated roots spread to observe various macroscopic changes and length measurement.

DISCUSSION

The pesticides inhibited the root growth dose dependently. It was observed that all the three pesticides showed decrease in root length with the increase in concentration. This shows the cytotoxic nature of the pesticides under study increases with the increase in concentration and justifies their degree of toxicity. Different pesticides have been found to have genotoxic effects on plants [25]. Parameters such as root growth can be used to estimate the cytotoxicity of environmental pollutants [26; 27. Fisksejo [23] and Chauhan and Sunderaraman [24] have given consideration for 5th day measurement of root lengths. It is shown that the roots of onions and various crop seedlings are most frequently used to obtain toxicity data by determination of root growth inhibition. Roots are chloroplast free, non-photosynthetic systems and therefore, with regard to their cytotoxic reactions are closer to vertebrate tissues and cells than are chloroplast-containing plant organs [28].

Mostly people spray pesticide formulations at least after a gap of 5 days. So the potential effect of a particular pesticide has to be evaluated after this minimum gap of five days to have a clear result of toxicity. The three pesticides in the present study showed a dose dependent decrease of root length and number indicating their toxic nature. The fact that the decrease in root growth over 45% strongly indicates the presence of toxic substances Fiskesjo [23], besides having lethal effects on plants [29]. *Allium cepa* root growth was inhibited by the pesticides in a dose dependent manner and any inhibition of growth reflects toxic effects on metabolic processes [30].

In five day root inhibition test of various concentrations, it was possible to estimate EC50 value of the pesticides in the present study. Since initially certain concentrations inhibited root growth completely and it is impossible to conduct genotoxic evaluation further. Therefore it is compulsory to know the concentration of a chemical which could yield required root length to be treated with a pesticide and carry out its genotoxic potential. In the present study, a range of pesticide concentrations were treated till a standard concentration range was carved out and EC50 value was established. The effective concentration (EC50) value proved to be useful parameter for selecting the test concentrations for the genotoxicity assays [31].

The EC50 values evaluated and reported in the present study can be used to determine appropriate pesticide concentrations of these three pesticides for further genotoxicity assays. Inhibition of rooting in the *Allium* test and the appearance of stunted roots indicate retardation of growth and cytotoxicty, while root wilting explains toxicity [32; 33]. Nevertheless both growth retardation and root wilting are accompanied by suppression of mitotic activity and occurrence of chromosomal aberration. Root growth inhibition in higher concentrations of these three pesticides provides evidence that higher concentrations of pesticides decrease cell division because of inhibition of mitotic index. The suppression of mitotic activity was often used in tracing cytotoxicity [34]. Amin and Migahed [35] have reported that suppression of mitotic index is often accompanied with the increase in cells with C-mitosis, sticky and abnormal orientation.

Nuvan exhibited maximum and remarkable cytotoxic potential followed by Endosri and Kvistin. The morphological abnormalities were observed in a high frequency in case of nuvan. It showed a high decreasing trend in number of roots compared to endosri and kvistin. The abnormalities in the growth of roots were in agreement with Fiskesjo [36], according to which the growing roots tips may, after various treatments, take the shapes of hooks, spirals or tumours. Such observations may give information of specific action of a chemical [37]. The induction of various morphological abnormalities like crochet hooks and c-tumors, which have been shown by other studies to be useful sign of toxicity [26]. According to Odeigah et al. [38] heavy metals may be cause of Crochet hooks while c-tumor effects are caused by several types of compounds such as colchicines. So here these pesticides act like heavy metals and colchicine like compounds in the induction of these root abnormalities. The results of the present study indicate that these chemicals can lead to toxicity in mammalian systems as well. Chauhan et al. [31] studied comparative effects of insecticides on *Allium* and mammalian test systems and reported that *Allium* test responds to these insecticides similar to mammalian test systems. Changes in roots like crochet hooks, C-tumors and broken roots were also reported by Ateeq et al. [39] in the root tip cells of *Allium* cepa after treatment of pesticides. Various hazardous compounds have resulted in crochet hooks, dark brown, pale and thin roots in *Allium cepa* [39-41]. Crochet hooks are a type of tumor where the elongation zone of the root develops a swelling (Olorunfemi et al. [40] and causes the root to bend upwards. These are signs of toxicity. The change in color in the present study by various concentrations of pesticide treated roots to brownish was due to cytotoxic effects causing cell death [42].

The reduction in the number of roots with the increase in pesticide concentration also indicated the relation of toxicity of pesticides with root inhibition. The higher concentrations produced fewer roots as compared to control. This may be due to high toxicity to few roots at their initial stage while few may exhibit resistance.

CONCLUSION

Since these pesticides not only remain restricted at the site of spray but get washed off with the runoff and reach the domestic water supplies, which could hold severe implications on the health of local citizenry. The effect of pesticides under study could lead to various afflictions in other organisms also as their toxic nature in root meristem cells is indicative of the fact that the present pesticides may cause deleterious effects and should be evaluated through other studies also. Also keeping in view the indiscriminate use of these three pesticides, there is a high chance of contamination of water and food used by people and other organisms. Exposure to chemicals through drinking contaminated water is capable of inducing DNA damage and enhancing genetic changes in somatic cells that can result in decreased cell survival or transformation and eventual reproductive abnormalities and cancer formation in organisms [43; 44].

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