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Allium Test: A Pioneer Method For Assessment Of Genotoxicity Of Pesticides

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ABSTRACT

For the screening of genotoxicity of pesticides various plant materials has been used as test material but common onion(*Allium cepa*) and garlic(*Allium sativum*) is an excellent plant for the biomonitoring of pesticides. The increase use of pesticides in agricultural field as well as in house pest control raise a question of interest that these pesticides causes any cytological damage or not. To screen out the effect of the pesticides the *Allium* test is a common and routinely used method. The mitotic and meiotic both the studies of *Allium* are held for the assessment of genotoxicity of pesticides. The inhibition in mitotic index and induction in chromosomal aberrations by the treatment of pesticides are the parameter of assessment of toxicity.
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KEYWORDS

Allium test;
Pesticides,
Genotoxicity,
Chromosomal aberrations.

INTRODUCTION

The term pesticide refers to chemical substances that are biologically active and interfere with the normal biological process of living organisms deemed to be pests. Pesticides can be broadly classified according to their intended target pest(i.e. Herbicide for weeds, insecticide for insects, fungicide for plant diseases and molds and so on). They are also characterized by chemical structure and properties. Pesticides are designed and intended to be biologically harmful to living organisms deemed to be pests. The biological activity of pesticides is achieved by different modes of action.

Toxicological studies are a cornerstone of the testing that aims to insure the risks posed by pesticides when they are used as intended are not unacceptable.

Interestingly India's consumption of pesticides

per hectare is low when compared with world averages 0.5 kg/ha against Korea 6.60 kg/ha and Japan's 12.0kg/ha. According to pesticides industry statistics, India spend $3 /ha on pesticides compared with $24 /ha spent by Philippines, $255/ha spent by South Korea and $633/ha by Japan .

Yet despite comparatively low use of pesticides in India, the contamination in food products in country is alarming. About 20% of Indian food products contain pesticides residue above tolerance level compared to 2% only globally. 40% of all pesticides used in India belong to the organochlorine class and 30% of the pesticides used belong to the organophosphorus[40].

The synthetic pyrethroids are used to protect crops animals and humans from a wide range of insects[30]. The use of synthetic pyrethroids got increased several folds in recent years due to there low mammalian toxicity and limited persistence in soil as compared to organochlorine insecticides. But synthetic pyrethroids are highly toxic to fish and other lower aquatic organisms[15,34] and there widespread use has led to toxic effects in plants animals and human beings. The variations in chemical structure causes variations in toxicity among individual pyrethroids insecticides[25].

Genotoxicity is a possible serious side effect of pesticide exposure. Previous studies indicate that individual pyrethroids, organophosphate and organochlorine insecticides increase number of chromosomal aberrations like micronucleus formation and chromosomal breaks in dividing cells of plant. A number of plant bioassay have been developed for the detection of environmental mutagens because plant chromosomes are relatively large and respond to treatment with mutagens in a similar way to mammals and other eukaryotes[21]. Among these assays, the *Allium* test is routinely used for studying the effects of toxic materials on chromosomes and cell division and has been recommended as a standard assay for environmental monitoring[18]. The common onion *Allium cepa* is an excellent plant for the assay of different chemicals in respect of chromosomal aberrations. Beside *Allium cepa Allium sativum* have also been used. The *Allium* test for assessment of genotoxicity was introduced by[31].

Protocols have been given for using root tips from either bulbs or seeds of *Allium cepa* to study the cytological effects of pesticides. The karyotype of *Allium cepa* has been described by[35]. They contain about 8 pairs of chromosome(2n=16). The duration of the mitotic cycle of *Allium cepa* has been varied at the different temperature[20,33,36]. Other plant material *Hordeum vulgare*, *Tradescantia*, and pulses are used for both mitotic and meiotic studies to test the mutagenic potentialities of chemicals. The mitotic studies of *Allium cepa* for assessment of mutagenicity is not only because of there effectiveness but also due to simple method of assessment as well as the experiments were carried out in lab condition. They are not requiring any special facility to grow. *Allium cepa* and *Allium sativum* has been used to study for the cytological point of view that follows the treatment and study their effects[3,28]. A large number of studies have been carried out with the help of *Allium cepa* and *Allium sativum* to assess the genotoxicity of different herbicides, insecticides and fungicides.

They used the seeds of *Allium cepa*[1]. When the root tips of seeds were 3-4cm long, the germinated seeds were treated with the mentioned concentrations of test chemicals.

Methods for treatment of *Allium*

Healthy uniform size bulb of onion *Allium cepa* of about 10-30 gram selected. Jette Rank (2003) used some special equipment. The glass tubes(Wallin glass) are bottom less and a 70mm ruler is mounted on the side of glass and then used to measure the length of root bundles. The beakers are made of polycarbonate and are disposable. This equipment is produced in his laboratory but assay can also be carried out by using normal test tubes or beaker. Prior to experiment the outer scales of bulb are peeled of and kept it under the running tap water for half an hour[7]. During experimentation for proper root growth bulbs are maintained in a B.O.D. incubator at $20+1^0$C in dark[10]. The bulbs are submerged in tap water to about one quarter the depth of bulb. Change the water daily. After approx 2-5 days at 20^0C the roots will grow to a length of 1-5cm. Approx 15-20 roots will be produced with in 5 days[22]. When the roots reached 1.5-2cm they were placed at different concentrations of test solution for the treatment of root tip cells[39] where as for the *Allium sativum* cloves of *Allium* were washed in running tap water for 30 minute. Sand

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and soil mixture suitable for the root growth of *Allium sativum* were prepared in the ratio of 2:1[4]. The growth is maintained at 20+2^{0}C in BOD incubator.

The test concentrations were determined by the EC 50 of that particular test chemical[41] or it may be referred as LD 50 i.e cytotoxic threshold of each chemical[5]. He also works on the recovery period by transfer of treated solutions on Hogland nutrient solutions[5]. They were left the treated seeds of *Allium* in distilled water for 24 hrs of recovery period[1]. The treated root tips are collected and fixed it either immediately or it may be treated with 0.025% to 1% of colchicine to arrest the chromosome in metaphase.

The 1-2 hrs treatment of colchicine is sufficient. The metaphase stage arrest due to because colchicine destroyed the spindle and prevents the mitotic cycle.

1. Cytogenetic assay

1.1 Fixation and storage

For cytogenetic studies the treated root tips are fixed in cold carnoy's solution[9] Carnoy's solution is the mixture of ethyl alcohol chloroform and acetic acid in 6:3:1 ratio respectively. Some workers also used the 3:1 ratio of acetic alcohol i.e. 3 parts of 95% ethanol a nd 1part glacial acetic acid in a fridge for over night[1,5,6,7,8,16,17,22,32,39]. After fixation, the material can be stored in 70% alcohol in refrigerator or at 4^0 C[5,6,22,39,44,49] maintained the root tips in 80% ethyl alcohol. Were fixed the root tips in propiono alcohol (1:2 v/v)[37].

1.2. Staining

The procedure to stain the root tips is feulgen squash technique given by[13], or by the method of[5,6,16,27,37,39,42,49] The staining procedure may carried out either with the help of 1-2% acetocarmine[1,29] or acetoorecine[1,17,38,43]. Some workers also used the hematoxyline to get better results. The different method to prepare the stain of hematoxyline is given by[24]. The traditional method to prepare the stain is dissolving 4 gram of hematoxylene and 1 gram of iron alum in 100ml of 45% acetic acid at room temperature. The mixture homogenized with a glass stick and kept in dark flask for 1 week. After the period of a week the mixture was filtered and kept it in a dark glass. The solution could be used immediately or stored for an undeterminate period in refrigerator.

He demonstrated that an aceto-iron hemato-xylene produced excellent results for chromosome analysis of plants[26,47]. try to simplify this technique working with a stock solution of 2% hematoxyline and 0.5% iron alum both in propionic acid. The stain may use in the concentration of 0.5% to 4% for better results with respect to plant material. The result of staining varies with the ripening time of stain[7,44,46]. To make the squash of root tips they are hydrolyzed with 1N HCL at 60^{0}C. for about 4-12 minutes (varies with plant material generally 5 minutes)[22]. If the plant material/root tips too hard it should treated with pectinase 5% for 1-3 hrs. long duration exposure of root tips with pectinase make problem to handle further. Make the squash of root tips in 45% acetic acid and mount with cover slip along with euparol and seal the slide by the sealer or rubber sealer. To make the permanent slide dry ice method of[12] or liquid CO_2 treatment method of[42] are used when it frozen the cover slip removed and slide immersed in absolute alcohol and mounted in euparol with a clean cover slip.

Analysis of genotoxicity (Data collection)

The genotoxic effects of pesticides are primarily at the mitotic index. To calculate the mitotic indexes use the following formula. The data of mitotic index for each class of treatment in comparison to control set of experiment shows the genotoxicity of treatment.

$$\mathbf{MI}=\frac{\mathbf{Number\ of\ cells\ in\ division}}{\mathbf{Number\ of\ cells\ observed}}\times 100$$

The decrease in value of mitotic index shows the genotoxicity of particular treatment. MI Less than 22% in comparison to negative control shows the lethal effect of sample on plant and animal system[2]. Secondary parameter to assay the genotoxicity is chromosomal aberrations seen in different treatment. It is calculated in percentage. The chromosomal aberrations found in *Allium* test indicates the effect of toxic chemicals that primarily affect the spindle apparatus, which has been reported with several chemicals[14].

Different types of chromosomal abnormalities are reported by different workers by the treatment of different types of pesticides(herbicides, insecticides and fungicides) like chromosomal breaks, micronucleus formation, binucleated cells, stickiness

of chromosome, laggards, chromosomal bridges may be one, two or three bridges along with laggards, vagrant, chromosome ring, asynchroyony, late separation, fragmentations.

They divided the aberrations of chromosomes in two categories i.e. mitotic aberrations and chromosomal aberrations on the stages of chromosomes[11].

The analysis of data and the significance of data are expressed statistically which shows the significance level of the results. The statistical analysis of data of mitotic index may done by the standard error[6,7] and the significance of aberrations are analyzed by the student's t-test[5,7,16,39] or by ANOVA test[41] or may analyzed by chi square test ($\chi 2$)[6,41,] (Jette Rank) 2003. He also adopt the z-test for analysis of data[6].

CONCLUSION

In the search of test systems which combined with the chemical analysis, can be used to provide data as a scientific basis for regulating the discharge of potentially hazardous substances into the environment and suitable for performance of toxicity evaluation, the *Allium* test seems to have some advantages because the *Allium* test is simple, sensitive, rapid bioassay, easy to handle, it has low cost and it shows good correlation with mammalian test system[18]. The chromosomes of *Allium* are relatively large and plant shows correlation with mammalian and non mammalian test system[22]. It provides useful estimate of the total toxic effect resulting from the treatment of root tip cells by the insecticides[19]. The *Allium* test also used to study the genotoxic effect of pollution of heavy metals in water sample, contamination of river water, lechates, hazardous solid wastes, sewage water and any other hazardous substances[8,23,27,45]. So this test of biomonitoring is a superior, less expense and excellent method which applied for the assessment of genotoxicity of pesticides or any other hazardous substances.

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