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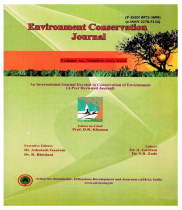

Assessment of the effects of fungicide (Thiram) on somatic cells of broad bean (*Vicia faba* L.)

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ABSTRACT

Present study was carried out to know the impact of fungicide (Thiram) on somatic cells of broad bean (*Vicia faba L.)*. Thiram is one of the most effective fungicide. Significant inhibition of mitotic index and increase in the frequencies of chromosome aberrations were observed. Results of the study indicate that both the plant bioassay found to be sensitive indicators for the genotoxicity assessment as the outcome of majority test system. Using plant bioassays for testing and monitoring environmental chemicals or pollution has many advantages. The mitotic index decreases due to exposure of plant extract with thiram in higher concentration as 150, 300, 550, 800 ppm for 8h and show reducing effect that is 21.00, 20.70, 18.61 and 16.77 in comparison to control that is 21.19. The higher concentrations of fungicide showed the genotoxic effects and damage the chromosomal integrity.

Introduction

Fungicide are the chemical substances, which is applied worldwide in public sectors and agriculture areas. Due to its high biological activity and in few cases of their persistence in the environment, the use of fungicide can lead undesired effects on animal health especially human and to the environment. On the other hand, fungicide play a significant role in rural health program comprises of the control of biting, noxious, irritating, annoying or contaminating insects and other pests, which infest human and animals (Crosby, 1981). Fungicide residues enters in food chain due to their indiscriminate use in public and agriculture areas. The residues of fungicides can be detected in different feed crops, food stuffs, flesh and meat, poultry, fish and in milk products. The modern agricultural practices represent a very large input of toxic chemicals in our environment (Crosby, 1981; Ajay and Sarbhoy, 1987). Chemical fungicides have contributed greatly to the increase of yields in agriculture by controlling diseases and also towards checking the diseases (Amer and Farah, 1974). The need to increase the production of food for the rapidly growing world population is a big challenge. Fungicides are used to control the population of pests. The biological activity of fungicide is attained by different mode of action. A large number of synthetic fungicides are under use. They belong to different chemical groups and are classified on the basis of their chemical structure and properties. Chemical structure of fungicide is a key to the action of these pesticides. Their usage has increased manifolds in disease control management without considering their harmful side effects on plants, animals and human beings (Ajay and Sarbhoy 1987). Although, the use of these chemicals has become essential, but their ingredients have induced acute toxic effects (Amer and Farah, 1974) (Badr and Elkington, 1982). The toxic effect of endosulfan is not necessarily a result of direct application, some pesticides accumulate into the food upto a toxic level and affect the public health (Dryanovska, 1987; Yadav, 1986; Cantor *etal*., 1992; Mehmet & Huseyin, 2017). *Vicia faba* L. (2n = 12), of the family Fabaceae, have homozygous genotype because of self-pollination (Gulfishan *et al.,* 2010). Thus, *Vicia faba* based bioassays have established role to study chromosome anomalies due to their large and visible chromosome (Asthana and Kumar, 2014;

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Zehring *et al*., 2022). Present study was carried out to know the impact of fungicide (Thiram) on somatic cells of broad bean (*Vicia faba* L.).

Material and Methods

Thiram is a fungicide and belongs to the ethylene bisdithiocarbamate (EBDC) chemical class. The fungicide of EBDCs class are used in agriculture area to prevent crop damage and to protect harvested crops from fungal infections during storage or transport. Thiram is a widely used, broad-spectrum fungicide and a metabolite. The roots of plants were cut off when the length of roots become 1.5-3.0 cm in length and then fixed in a chemical acetic acid-ethyl alcohol (1:3) V/V. After that the process of hydrolysis is done in 1N HCL. At last, the sample/replicate is stained by using Feulgen squash technique. For each treatments and control, three replicates were used and examined. The experiments were done in laboratory at normal room temperature (22±2ºC).

The mitotic index and the mitotic inhibition were calculated according to the standard formula. Mitotic Index (MI) and chromosomal aberrations (CA) in mitotic cells frequency were scored from the slides observation. Many cells were observed to score the mitotic index and chromosomal aberrations in each concentration. All observations were taken from temporarily prepared slides. In each sample, many cells were observed to record data of mitotic index and different chromosomal aberrations (Yuzbasioglu *etal*., 2003).The formula for calculating mitotic index and abnormality percentage are :-

Mitotic Index (MI %) = Total No. of dividing cell / Total No. of cells observed X 100

Results and Discussion

Present study was carried out to know the impact of fungicide (Thiram) on somatic cells of broad bean (*Vicia faba* L.). Thiram is one of the most effective fungicide. Significant inhibition of mitotic index and increase in the frequencies of chromosome aberrations were observed. Results of the study indicate that both the plant bioassay found to be sensitive indicators for the genotoxicity assessment as the outcome of majority test system. Using plant bioassays for testing and monitoring environmental chemicals or pollution has many advantages. The mitotic index decreases due to exposure of plant extract with thiram in higher concentration as 150, 300, 550, 800 ppm for 8h and show reducing effect that is 21.00, 20.70, 18.61 and 16.77 in comparison to control that is 21.19. The higher concentrations of fungicide showed the genotoxic effects and damage the chromosomal integrity (Table 1).

Table 1: Effects of fungicide on mitotic index in root meristem of Faba bean (*Vicia Faba)* after 8h exposure

Treatment	Concentration (PPM)	Mitotic Index
Control	0	21.19
Thiram	150	21.00
	300	20.70
	550	18.61
	800	16.77

Thiram affects the duration of each mitotic stage of each replicate as compared to control. It also play an important role in reducing mitotic index, indicating mitotic inhibition and increase in the frequency of abnormal mitosis, significantly. Star metaphase was one of the chromosomal abnormalities observed. Such type of abnormality was also observed after treatment of *Vicia faba* L. root tips with Thiram and considered as being a fore step of disturbance of the spindle completely. The effect of Thiram on root mitosis stimulates that of colchicine in the type of abnormal metaphase and anaphase and the induction of polyploidy cells as well as accumulation of metaphases. The threshold dose (dose of pesticides on which they retain their fungicidal property but have little or no cytotoxic/ genotoxic effect. The result show that the metaphase stage in treated root tip cells (*Vicia faba)* was also the most influenced stage by extract treatment and the total percentage of its abnormalities was higher than other mitotic stages. In this respect, abnormalities in the other mitotic stages was observed in the following sequence as Prophase> anaphase>telophase for all replicates / treatments. The study revealed that Thiram had a detrimental effect on the test material, there was inverse relation between the mitotic index and the dosage and time of treatment, and the direct relation between the mitotic index and the dosage and the time of treatment of percentage of abnormalities,

treatments not only brought down the frequency of dividing cells, but also produced a good number of anomalies in the mitotic cells. There was a notable decrease in the mitotic index and gradually increase in the chromosomal abnormalities as the concentration of the experimental solution and the time of treatment increased. The cytotoxic effects of fungicide were assessed employing chromosomal aberration bioassay in root tip cells. Different cytological aberrations viz., non-orientation and mis-orientation of chromosomes and stickiness, bridges, preconscious movement, laggards and fragments, diagonal spindle formation were observed in increased frequency with increasing concentrations of fungicides. These abnormalities have also been reported for several extracts and chemicals already observed by many workers (Yadav, 1986; Nwakanma *et al.,* 2009; Mohamed and El-Ashry, 2012; Yuzbasioglu *et al.,* 2003).

Conclusion

Finally, this work provides the information about the effect of fungicide Thiram on plant cells. Results of the study indicate the genotoxicity of Thiram as compared to biopesticides. According to the earlier study, the biopesticide also affect the cells but not significantly. So, conclusively we can say that the biopesticides are safer alternative as compared to chemical pesticides. Increasing use of agricultural chemicals for improving the field crops is now in routine use. Now a day, biopesticides are achieving a modicum of growth as alternative to conventional pesticides. However, their full potential has yet to be reached.

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Conflict of interest

The authors declare that they have no conflict of interest.

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