



RESEARCH ARTICLE

COMPARATIVE STUDY OF CYTOTOXIC EFFECT BY INDOFIL, BAVISTIN AND BIOFUNGICIDE ON
THE SEEDS OF LENTIL (*LENS CULINARIS* M.)

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ABSTRACT

The effect of three fungicides namely Indofil, Bavistin and biofungicide on mitotic index, chromosomal abnormalities, seed protein content, radicle-plumule length variation on lentil (*Lens culinaris* Medik) were assessed through a seed treatment experiments. Seeds treated with different doses (0.5%, 1%, 2%) of the three fungicides (above mentioned) solution along with control set. The systemic fungicides (indofil & bavistin) treated seeds have the ability to induce mitotic abnormalities in large number at 2% concentration, in contrast to biofungicide, which is less toxic. However, the plumule-radicle length is highest in case of seeds treated with 0.5% bavistin & indofil and with 1.0 % in bio fungicide. The endosperm weight decreases gradually in respect to total plumule-radicle weight. In general, the toxicity of fungicide in terms of chromosomal abnormality increases as follows: indofil >bavistin >biofungicide. From the proposed study, it can be concluded that Bio fungicides are more safe than systemic fungicides as they posed very negligible effect on cell division and chromosome profile.

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INTRODUCTION

Lentil (*Lens culinaris* Medik) is one of the important members of Leguminosae (Papilionaceae) with chromosome number $2n=14$. It is a major grain legume crop in many developing countries like West Asia and North Africa and many other area of the world. Lentil can grow well also in arid to semiarid climate (Mojein *et al.*, 2003). In India it is now cultivated in Andhra Pradesh, Maharashtra, Madhya Pradesh, Uttar Pradesh, Rajasthan and West Bengal. The Lentil is a twining, slender, much branched annual herb with tendrils. The pods are short and broad, with small lens-shaped seeds. Seed color ranges from yellow to red-orange to green, black. The seeds are mainly used as dal; and soups. Lentil seeds contain huge amount of proteins as compared to other crops and occupy 3rd rank after soyabean and hemp on the basis of the content of seed protein. Generally wild varieties of lentil are resistant to stress. But today cultivated lentil varieties are prone to various diseases due to biotic stress such as Rust (*Uromyces fabae* Grev. Fuckel) and powdery mildew (*Erysiphe polygoni* DC.), which appear naturally in epidemic form in North West Asia (Gupta *et al.*, 2005), interfering with the yield potential subsequently leading to huge loss of economy. Foot rot (caused by *Fusarium oxysporum* and *Sclerotium rolfsii*) is considered as an important and destructive disease of pulses in

almost all legume-growing countries of the world. The fungicides highest performance was found with Secure 600wg (0.2%) in controlling the incidence of foot and root rot (Hoque *et al.*, 2014). Fungicides are most commonly used against fungal diseases of agricultural crops in many countries of the world. Although fungicide application results in rapid control of the diseases, the widespread use of these chemicals may cause environmental and food contaminations (Fisun and Rasgele, 2009; Tort and Turkyilmaz, 2003). Indofil M-45 is a broad spectrum, protectant and contact fungicide popularly known as KING OF FUNGICIDE. Mancozeb (indofil M-45) has a molecular formula $(C_4H_6MnN_2S_4) \times Zn$. It is effective against diseases caused by fungal pathogens in various crops like Paddy, Potato, Tomato, Chili, as well as some fruits, vegetable, cereals and pulses. Bavistin is a systemic, broad-spectrum fungicide with protectant and eradicant activity used to control fungal diseases. Biofungicides are microorganisms and naturally occurring substances that control diseases. Residues of fungicides can lead to environmental pollution and have adverse effects on people and animals. Besides this, the application of such chemicals in agricultural plants has harmful effects on their cytological profile (Cali, 2008).

MATERIALS AND METHODS

The Seeds of *Lens culinaris* Medik var. Asha and fungicides were procured from Krishi Progoti Bolpur west Bengal. The fungicide selection was purely on the basis of the frequent use in the Agricultural fields by the

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Table 1. Change of plumule – radicle length after treatment with fungicides at different concentration

Types of fungicides	Concentration	Plumule length (cm)			Radicle length (cm)		
		4 th day	8 th day	12 th day	4 th day	8 th day	12 th day
Indofil	control	4.67±0.37	6.00±0.49	9.10±0.24	3.42±0.37	4.05±0.33	4.10±0.67
	0.5%	2.95±0.27	9.42±0.74	12.52±0.89	2.52±0.31	3.87±0.41	3.87±0.85
	1%	2.80±0.36	8.75±0.71	10.15±0.84	2.35±0.15	2.85±0.42	3.20±0.69
Bavistin	2%	2.67±0.22	6.02±0.20	8.80±0.18	2.42±0.13	4.37±0.33	4.67±0.13
	0.5%	2.90±0.44	6.35±0.82	9.97±0.90	2.97±0.20	3.72±0.36	3.75±0.49
	1%	2.57±0.22	6.07±0.83	8.82±0.21	2.72±0.07	3.07±0.33	4.25±0.81
Bio fungicide	2%	2.17±0.12	5.85±0.33	7.77±0.89	2.50±0.16	2.67±0.53	2.90±0.49
	0.5%	3.85±0.23	8.6±0.50	10.75±0.85	4.40±0.22	4.07±0.60	4.92±0.25
	1%	4.00±0.07	9.62±0.54	13.85±0.39	3.90±0.12	6.55±0.88	6.42±0.41
	2%	3.15±0.15	8.75±0.65	8.75±0.35	4.07±0.41	3.95±0.67	4.37±0.71

Table 2. Mitotic index (MI), type and percentage of mitotic abnormalities on root tip cells of *Lens culinaris M.* exposed to fungicides

Name of fungicides	Concentration	Mitotic index (Mean±SE)	Mitotic abnormalities %					Total abnormalities %	
			S	F	Cl	P	C-M		S-T
	Control	7.82±0.74	0	0	0	0	0	0	0
Indofil	0.5%	6.34±0.27	0	2.87	2.87	0	0	2.87	8.44±3.80
	1%	6.02±0.24	3.2	0	6.4	3.2	0	0	12.90±3.49
	2%	5.68±0.41	6.06	9.09	12.12	0	6.06	0	33.33±3.96
Bavistin	0.5%	5.90±0.56	0	0	5.7	0	0	0	5.71±3.36
	1%	4.18±0.33	3.4	3.4	3.4	0	0	0	10.20±3.89
	2%	3.26±0.32	0	4.5	9.0	4.5	0	0	18.18±5.58
Biofungicide	0.5%	7.30±0.40	0	0	2.7	0	0	0	0.0
	1%	7.36±0.63	0	0	0	0	0	0	2.7±1.1
	2%	6.48±0.60	0	0	5.1	0	0	0	5.10±2.70

Abbreviations: S: Stickiness; F: Fragmentation; Cl: Clumping; P: Pycnosis; C-M: C-mitosis; S-T: Star-Telophase

farmers of Birbhum region. The concentrations were determined on the basis of their dosage used in agricultural fields. Different concentrations (0.5%, 1%, 2%) of fungicides were prepared along with control (treated with tap water). All experiments were done in three replicates and for each replicate 30 seeds were imbibed in treatments for 24 hours in petridishes lined with wet blotting papers and allowed to germinate at 18±8°C in light for 10 hrs and at dark period for 14 hrs. The changes in radicle - plumule length, for each concentration and for each replicate were recorded after an interval of 4th days, 8th days & 12th day. The total weight of radical - plumule against the total weight of endosperm were also recorded. The standard deviations and standard errors have been calculated for each observations. The study of mitotic index, roots tips of lentil after growing to certain length (2 mm) were fixed in acetic – ethanol (1:3) and stained according to aceto - orcein squash procedure, followed by the methods described by Sharma and Sharma (1980). To determine the effects of these chemicals on mitotic index, 600 cells were scored in the control group and also in each treated group, viewing under microscope (40X) and mitotic index and abnormality percentages were calculated as follows:

$$\text{Mitotic index} = \frac{\text{total number of dividing cells}}{\text{total number of cells present per microscopic field}} \times 100$$

$$\text{Mitotic abnormalities} = \frac{\text{Total no of abnormal cells per microscopic field}}{\text{Total no dividing cells present on that microscopic field}} \times 100$$

RESULTS AND DISCUSSION

It was observed for all the cases that endosperm weight started to decrease along with the increase in total plumule radicle weight day after day. The Table 1 infers that fungicide treatment has resulted in drastic decrease in radicle as well as

plumule length with increasing concentrations. However among three groups of fungicides, biofungicides were more favourable for increase in length of radicle and plumule than indofil followed by bavistin. However radicle length remains more or less constant or increase at a slow rate for all the dosages. The radicle- plumule length increased more than control at 0.5% dosage of indofil and bavistin may had no adverse effect on radical - plumule growth where as 2% dosage may had adverse effect on radical- plumule growth, as it reduced the length. It can be concluded that indofil and bavistin at higher concentrations were more toxic than biofungicide because the systemic fungicides have more negative role upon mitotic index by reducing cell division with increase in number of days.

The Table 2, recorded the mitotic index and the chromosomal aberrations of root tip cells of *L. culinaris* after 24 hrs treatment with indofil, bavistin and biofungicide along with control. The maximum value of mitotic index was observed in control (7.82%) without any chromosomal aberrations. It was found that there was concentration dependent decrease of mitotic index at various concentrations like 0.5%, 1% and 2% compared to the control. In case of indofil treated seeds, the mean mitotic index decreased from in 6.34% to 5.68% at dosages 0.5% to 2% respectively. The bavistin treated seeds also recorded decreased percentage of mitotic index less than indofil. But in case of biofungicide the change in mean mitotic index was very little even at higher dosage. Mitotic index is used as an indicator of adequate cell proliferation biomarkers that measures the proportion of cells in the M-phase of cell cycle and its decrease could be interpreted as cellular death or delay in cell proliferation kinetics (Rojas *et al.*, 1993). The fungicides indofil and bavistin are widely used to control fungal diseases in lentil and

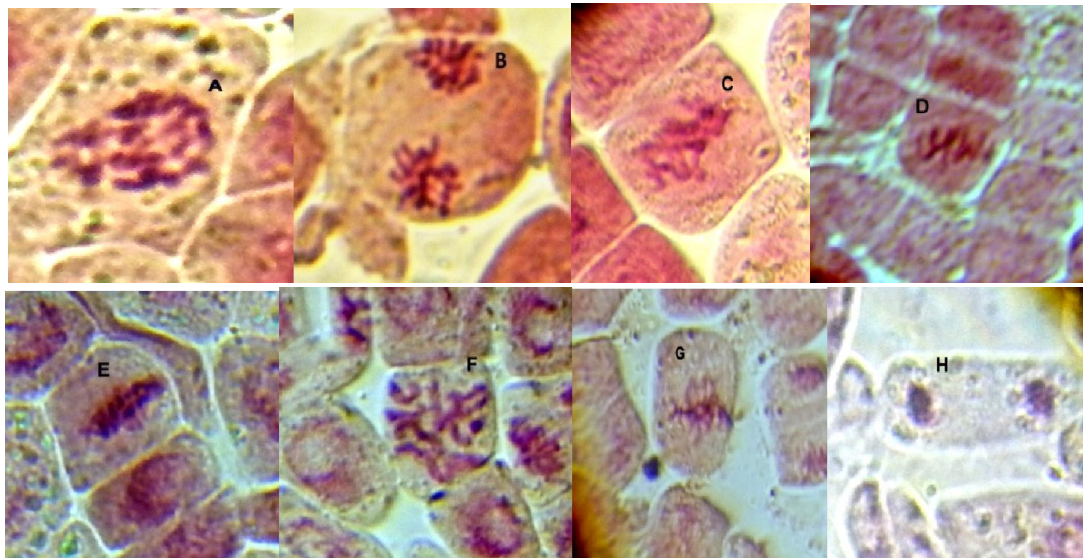


Figure 1. Normal and abnormal stages of Mitosis of *Lens culinaris* M. root tip cells treated with fungicides A-H: A: Pycnosis; B: Star-Telophase; C: Fragmentation; D: Stickiness; E: Clumping; F: C-Mitosis; G: Normal Metaphase; H: Normal telophase

other crops, but these may have cytotoxic effect in plant system. Mitotic index is an acceptable measure of cytotoxicity for all living organisms (Smaka-Kinel *et al.*, 1996). The cytotoxicity level can be determined by the decreased rate of mitotic index. The present cytological observation showed that indofil and bavistin both decreased the mitotic index at all dosages compared to control. The Chromosomal aberrations were observed in the treated root cells at different concentrations of fungicides. The ratio of abnormalities increased parallel to the increase in concentration of fungicides (Figure 1). This showed positive correlation of aberrant cells with the higher concentration. Among the three fungicides indofil showed highest number of aberrant cells compared to the other applied fungicides. Occurrence of Metaphase clumping, fragmentation, pycnosis and stickiness types of chromosomal aberrations were recorded in Bavistin treated seeds. Indofil treated seeds also showed all the four abnormalities along with c-mitosis, star - telophase Whereas Biofungicide showed only metaphase clumping at 2%. The fungicide Bavistin (Carbendazin) at 10 ppm concentration was the best among the treatments of *Cicer arietinum* while in case of *Zeamays* 1 ppm concentration of Bavistin has shown better stimulating effect on seed germination and plant growth (radical/ plumule) as compare to control (Dhanamanjuri *et al.*, 2013).

In the present study most common chromosome abnormalities were metaphase clumping, stickiness and fragmentation. Other than these star telophase, c-mitosis, pycnosis were also observed. The stickiness has been attributed to the improper folding of chromosomal fibers, which makes the chromatids connected by subchromatid bridges (Bard and Ibrahim 1987). Gauden (1987) postulated that sticky chromosomes result from the defective functioning of one or two types of specific non-histone proteins involving chromosome organization which are needed for chromatid separation and segregation. The altered functioning of these proteins is caused by mutation in the structural genes coding for them or by the direct action

of mutagens (Turkoglu, 2007). Disturbed metaphase and telophase may be due to disturbance of spindle apparatus which allows the chromosomes to spread irregularly over the cell; resulting in c-mitosis and star-telophase respectively (Amer and Ali, 1974). A decrease of mitotic index below 50% usually has lethal effects (Panda and Sahu, 1985). If mitotic index decreases below 22% of control, it causes sub lethal effects on the test organism (Antonsie-Wiez, 1990). The aberrations may suggest that fungicides were potent spindle inactivator which could cause metabolic disbalance. As the chromosome aberrations are indicator of genotoxicity, it can be concluded that indofil and bavistin posses more genotoxic effects than biofungicide in root meristem cells of *Lens culinaris* M.

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