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EFFECTS OF FUNGICIDE ON KARYOMORPHOLOGY OF *LYCOPERSICON ESCULENTUM* MILL.

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Abstract

Effects of fungicide on karyomorphology of three germplasm of *Lycopersicon esculentum viz.* Acc. No. BD-7748, 7749 and 10125 were investigated. All the germplasm treated with different doses of fungicide Ridomil Gold showed different karyomorphological features. Interphase nuclei of these germplasm with different doses were grouped in "Complex chromocenter type". Prophase chromosomes were "Gradient type" in all the germplasm treated with different doses. All the germplasm were found to possess 2n = 24 chromosomes. Ridomil Gold had no effect on chromosome number. Seeds treated with water (control) showed much better morphological features whereas different doses of fungicide showed worse result. Therefore, the present investigation showed that fungicide Ridomil Gold has negative effects on karyomorphology of *L. esculentum.*

Introduction

Tomato (*Lycopersicon esculentum*) belongs to Solanaceae is one of the popular winter vegetables in Bangladesh (Knapp *et al*. 2004). The domesticated tomato is cultivated for its well-liked fleshy fruits, which are known by a variety of names such as cherry tomato, grape tomato, roma tomato, heirloom tomato, green tomato, etc.

In daily life, tomatoes are frequently consumed and are a good source of antioxidants which has been linked to many health benefits, including reduced risk of heart disease and cancer (Hedges and Lister 2005). Tomatoes contain water and calcium. Because of its great nutritional content, it offers a balanced source of the vitamins A and E. But this crop is susceptible to bacterial, viral, nematode and fungal diseases. Application of fungicides can improve genetic potential and reduce yield loss brought on by disease.

Though it is very important for the control of several fungi, uncontrolled and widespread use of fungicides frequently causes serious environmental issues in addition to harming the health of consumers and may induce mutation (Sahu *et al*. 1981). Some are suspected to be even carcinogenic (Burnett *et al*. 1980). On the other hand, most of the fungicide decompose slowly and causes toxicity to the biosphere. Sometimes farmers in our country don't maintain the appropriate doses of fungicide which may leads to deleterious alteration in cell division, cell enlargement, chromosomal aberration and tissue differentiation. Several morphological changes and change of root length, shoot length can be detected due to excessive doses of fungicide. Even chromosome volume can also be decreased due to excess use of fungicide which may results in genetic variation (Bennett 1971).

So, it is urgent to minimize the use of chemicals and apply appropriate concentration for controlling disease and to know the karyomorphological effect in local conditions.

Therefore, the aim of the study was to find out morphological and cytogenetical changes due to different doses of fungicide.

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Materials and Methods

For the determination of karyomorphological features, seeds of different germplasm of tomato (*Lycopersicon esculentum*) *viz.* BD-7748, BD-7749, BD-10125 were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. This experiment was conducted with Ridomil Gold with two different doses. The fungicide was collected from the local market of Nama Bazar, Savar.

Two different doses were prepared by mixing 5 gm and 10 gm of Ridomil Gold in two beakers and their volume were made 1000 ml by adding distilled water. In these cases the above mentioned doses from lower to higher concentrations were designated as D_1 and D_2, respectively and the control was designated as C. Fresh seeds of *L. esculentum* were pre-soaked in two different concentration of Ridomil Gold. After 6 hours the solutions were decanted off and all seeds were washed thoroughly with tap water. The control (C) seeds were pre-soaked in distilled water for the same time. Half of the seeds were used for the germination purposes in the petri dish in laboratory at room temperature. Another half was sown in pots with 2-3 cm in depth.

The maintained area was mined for roots. A sharp blade was used to cut young, healthy roots between 0.5 and 0.10 cm from the tip. The collected root tips (RTs) were pre-treated with 8-hydroxyquinoline (0.002 M) for 30 min at room temperature after being soaked on a filter paper to remove surface water. For 15 minutes at 4°C, root tips were fixed in 45% acetic acid. The pre-treated RTs were hydrolyzed in a solution of 1N HCl and 45% acetic acid (2:1) for 20 seconds at 60 °C. The hydrolyzed RTs were then placed in a solution on filter paper and transferred to a clean slide. Now cut the meristem region with a blade. After adding 1% aceto-orcein, the sample was placed in an acetic acid chamber for 20 - 25 minutes. On top of the material was a fresh cover glass. Slides were then examined using a compound microscope (Cmos EU 2050890).

Results and Discussion

Germination percentage of treated seed of all the germplasm in both the doses were low in comparison than that of control and showed gradual reduction of germination percentage with the increased doses of Ridomil Gold. The germination percentage was inversely proportional to the doses (Table 1). Days required for germination was decreased with the increased doses of fungicide. Dose-2 of all germplasm required maximum days for germination (Table 1).

Table 1. Percentages of seed germination of *L. esculentum* treated with two different doses of fungicide.

Acc. No	Control %	Dose - 1 (5gm/l) %	Dose – 2 (10gm/l) %
BD-7748	77 – 85	62 - 65	40 - 45
BD-7749	80 – 90	55 - 60	35 - 40
BD-10125	85 - 95	60 - 65	45 - 50

Morphological observation on the effect of Ridomil Gold were recorded on some characters like root length (cm), shoot length (cm) at seven days after germination, plant height (cm) at 30 days after germination, flowering time after 45 days, fruit size at 60 days and fruit maturity at 75 days after germination.

In this experiment data on root length (cm), shoot length (cm), plant height (cm), flowering time, fruit size and fruit maturity indicated that Ridomil Gold decreased all of the characters and delayed flowering time of all the germplasm in dose-2 (10gm/l). On the other hand, seeds of all germplasm treated with water increased root length (cm), shoot length (cm), plant height (cm), flowering time, fruit size and fruit maturity (Table 2). Kumari *et. al*. (2009) reported that

Tetracycline, Streptomycin and Amoxicillin were found to be most effective, exhibiting reduction in root and shoot length in *Pisum sativum.* Similar results were also obtained by Patwary *et. al.* (1989) when the seeds of *Triticum aestivum* L. were treated with a chemical Bidrin.

Mitotic cell division's interphase nuclei and prophase chromosomes' staining characteristics typically offer karyomorphological traits that aid in differentiating between specimens. According to Tanaka (1971), several large heterochromatic regions were found scattered throughout the nuclei of three *L. esculentum* germplasm in this study, which could be categorised as "Complex chromocenter type", based on the nature of heterochromatin's staining properties. Ridomil Gold doesn't show any negative effect on heterochromatin nature in interphase nuclei of tomato. The prophase chromosome of all the germplasm in *L. esculentum* were darkly stained at one end and gradually become faint towards another end. According to Tanaka (1971), this prophase chromosome considered as "Gradient type". Usually, "Complex chromocenter type" of interphase nuclei showed "Gradient type" of prophase chromosomes. In this study, all the germplasm of *L. esculentum* followed the general rule. In *L. esculentum*, a nucleolus was found in interphase nuclei and prophase chromosome of Acc. No BD-7748 and 7749 in dose-1 and 2 (Figs 1b, 1c, 2b, 2c, 4b, 4c, 5b, 5c). The present result indicated that the Ridomil gold has no negative effect on prophase chromosome of tomato. These characteristic features of interphase nuclei and prophase chromosome were not found in the available literatures.

Table 2. Shoot length (cm), root length (cm) and plant height (cm) of *L. esculentum* treated with two different doses of fungicide.

Acc. No.	Shoot length (cm)			Root length (cm)			Plant height (cm)		
	C	D-1	D-2	C	D-1	D-2	C	D-1	D-2
BD-7748	5.73	3.5	2	2.14	1	0.5	25	16.5	12
BD-7749	6	3	1.5	3.9	1.5	0.9	22	15	10.5
BD-10125	7	3.5	1.2	4.9	2	1.5	30	18	15.5

All the germplasm of *L. esculentum* with different doses and control (BD-7748, 7749 and 10125) were found to possess (2n = 24) same somatic chromosome number which correlates with earlier studies (Figs 7-12, Table 3). The fungicide chosen for this study was unable to change the number of tomato chromosomes.

The total length of somatic chromosome complement was lowest (22.57±1.24 µm) in dose-2 of Acc. No. BD-7748 and highest (36.34 ±0.44 µm) for BD-7748 in control of same accession of *L. esculentum* was observed. On the other hand, 33.29±1.56 µm, 28.82±1.48 µm and 27.19±1.22 µm was observed for BD-7749 in control, dose-1 and dose-2, respectively. In case of BD-10125, 28.95±1.33 µm and 23.94±1.65 µm was observed in dose-1 and dose-2, respectively (Table 3).

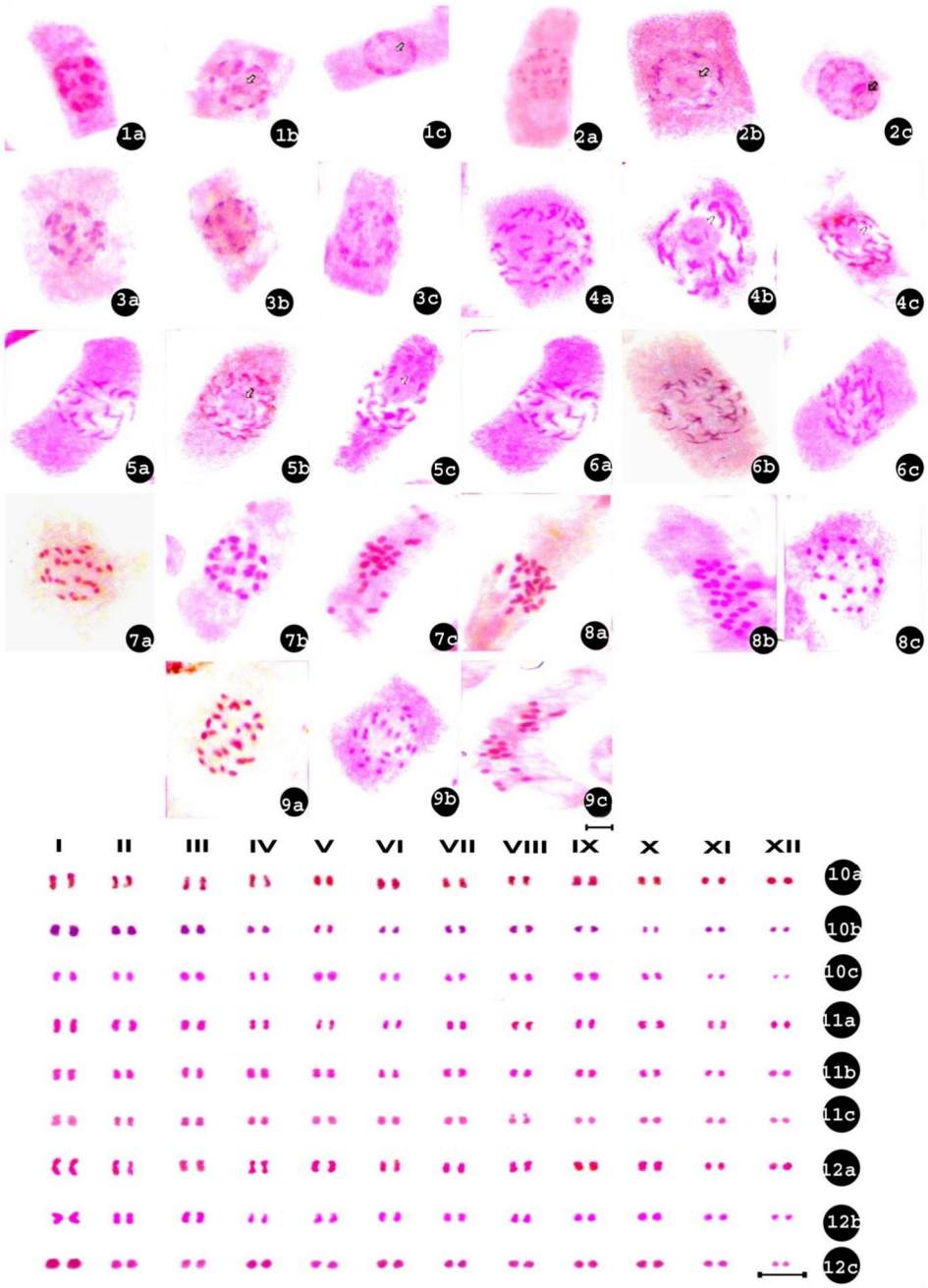
Among the three germplasm of *L. esculentum*, smallest average chromosome length (0.56 µm) was found in BD-7749 with dose-2 and highest (1.02 µm) in BD-10125 with control and dose-1were found (Table 3). Whatever, the three germplasm of *L. esculentum* could be characterized with the above cytogenetical features.

The relative length of all germplasm ranged from 0.02 to 0.06 (Table 3). There was some degree of similarity between the studied germplasm and this karyotypic parameter. There was no prominent effect of Ridomil Gold in any accessions of *L. esculentum*.

Table 3. Comparative karyotype analysis in three germplasm of *L. esculentum* after staining with orcein.

CP	Acc. No / Identity								
	BD-7748			BD-7749			BD-10125		
	C	D-1	D-2	C	D-1	D-2	C	D-1	D-2
2n	24	24	24	24	24	24	24	24	24
TCL (µm)	36.34 ± 0.94	27.28 ± 1.52	22.57 ± 1.24	33.29 ± 1.56	28.82 ± 1.48	27.19 ± 1.22	36.31 ± 1.27	28.95 ± 1.33	23.94 ± 1.65
RCL (µm) ($\bar{x}$ ± SD)	1.93 ± 0.02-1.03 ± 0.01	1.48 ± 0.10-0.76± 0.06	1.25±0.06-0.60±0.02	1.86± 0.10-1.02± 0.03	1.55± 0.10-0.86± 0.04	1.43± 0.08-0.72± 0.05	2.07± 0.06-1.05± 0.02	1.74± 0.06-0.72± 0.03	1.27± 0.06 -0.68± 0.10
ACL (µm)	0.90	0.72	0.65	0.84	0.69	0.56	1.02	1.02	0.59
RRL	0.03-0.05	0.03-0.05	0.03-0.06	0.03-0.06	0.03-0.05	0.03-0.05	0.03-0.06	0.02-0.06	0.03-0.05
DRL	0.02	0.02	0.03	0.03	0.02	0.02	0.03	0.04	0.02
RCI	28.42 -50.01	24.84-45.36	18.75-38.12	28.66 -49.68	22.33-44.25	17.15-37.50	30.63 -49.22	28.12-45.50	20.45-40.10
CF	14m +10sm	20m+4sm	24m	22m+2sm	20m+4sm	20m+4sm	16m+8sm	20m+4sm	24m
Syi%	62.34	54.12	80.25	49.12	51.15	51.12	59.25	52.2	80.25
AsK%	39.12	50.35	35.13	56.35	49.2	49.5	48.75	43.6	35.13
TF%	38.1	45.15	59.75	50.88	48.85	48.88	40.75	47.8	59.75

m = metacentric, sm = sub-metacentric chromosome.



Figs 1a-12c. Orcein-stained mitotic interphase nuclei, prophase, metaphase-chromosome and karyotype of three germplasm of *L. esculentum*.1a-3c. Interphase nuclei of 7748, 7749, 10125 in control, Dose-1 and Dose-2. 4a-6c. Prophase chromosome of 7748, 7749, 10125 in control, Dose-1 and Dose-2. 7a-9c. Metaphase chromosome of 7748, 7749, 10125 in control, Dose-1 and Dose-2. 10a-12c. Karyotype prepared from orcein-stained mitotic metaphase chromosome of 7748, 7749, 10125 in control, Dose-1 and Dose-2. Bar = 5 µm.

Out of three germplasm, all metacentric chromosomes were found in BD-7748 and 10125 in dose-2. In contrast, few metacentric and submetacentric chromosomes were observed in rest of the germplasm in control, dose-1 and dose-2. This feature indicated moderately symmetric nature of their karyotype.

In the present study, TF% ranged between 38.1 and 59.75%. Highest TF% was found in both the BD-7748 and 10125 in dose-2 (Table 3), representing strictly symmetric nature of karyotype which was correlated with its' chromosomal formula. In contrast, lowest TF% (38.1%) was found in BD-7749 in control, representing moderately symmetric nature of karyotype which was correlated with its' chromosomal formula (14m+10sm). Other value also indicated their moderately symmetric nature of karyotype. The reason of these karyomorphological changes might be due to chromosomal aberration.

Karyotype symmetry index (Syi %) was lowest (49.12%) in case of BD-7749 in control. Highest karyotype symmetric index value (80.25%) was found in both BD-7748 and 10125 in dose-2 (Table 3). With more asymmetry, the karyotype symmetry index values decreased. On the other hand, karyotype asymmetry index was lowest (35.13%) in both BD-7748 and 10125 in dose-2 and highest (56.35%) for BD-7749 in control of *L. esculentum* (Table 3). The value of asymmetric index (AsK%) increased with the increasing asymmetry. Ridomil Gold showed some effect on karyotype symmetric index, karyotype asymmetric index and total form. However, no report is available on these cytogenetical parameters in Bangladesh even abroad. These features are so much important for characterization of these germplasm and considered as a salient features of *L. esculentum.*

However, present investigation showed that the Ridomil Gold has negative effects on its germination percentage and morphology but did not found prominent negative effect on cytology.

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