COMPARATIVE STUDY BETWEEN THE EFFECTS OF THE SYNTHETIC FUNGICIDE MANCOZEB AND THE BIOLOGICAL FUNGICIDE PLANT GUARD ON *Allium cepa* PLANT

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Pesticides constitute a heterogeneous category of chemical specifically designed for the control of plant diseases. Pests cause annually a drastic loss of the economical crops allover the world. The wide production and use of chemical pesticides causes a serious pollution to the surrounding environment. These chemicals, a part from affecting the target pest, also affect economical plants, animals and human. Many investigators studied the mutagenic potentialities of pesticides using different systems (Njagi and Goplan, 1981; Datta *et al.*, 1995; Barakat, 1997; Biscardi *et al*., 2003; Bolognesi, 2003; Dane and Dalgic, 2005; Kaymak and Muranli, 2005; Singh, 2007). Higher plants provide valuable genetic assay systems for screening and monitoring of genotoxic agents and have recognized as excellent indicators of mutagenic effects (Grant and Owens, 2002 and 2006). *Allium cepa* and *Vicia faba* chromosomal aberration bioassay is an efficient and reliable short-term bioassay for the rapid screening of chemicals for clastogencity (Ma, 1982; Grant 1982 &1994; Kanaya *et al*., 1994; Abdel Migid *et al*., 2007).

Man today is concerned very much with the pollution of his environment. The field of environmental mutagenesis still needs more efforts in order to evaluate many thousands of pollutants that are released every day in our environment. Biological control agents for plant disease are currently being examined as alternative to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts. Species of *Trichoderma* and *Bacillus subtilis* have been used as effective biological control agents against some diseases. *Trichoderma* are commercially applied as biological control agents based on the production of some metabolites such as trichothecin, nonanoic acid and the enzyme α-1,3 or 1-6 glucanase (Antonia-Gallo *et al*., 2004; Madhu-Aneja *et al*., 2005; Montero *et al*., 2005; Sanz *et al*., 2005). Also, a cyclic lipopeptide compound (iturin A) produced by *Bacillus subtilis* has strong antifungal properties and low mammalian toxicity

(Klich, *et al*., 1994; Nagorska *et al.,* 2007).

The present investigation was planned to compare the effect of the synthetic fungicide mancozeb with the biological fungicide plant guard on the mitotic activity and percentage of different parameters of the cell cycle in root tips of *Allium cepa.* Also, the capacity of these fungicides to induce chromosomal aberration and change in seed protein electrophoretic profiles were investigated.

MATERIALS AND METHODS

Cytological experiment

Bulbs of *Allium cepa* plant variety Giza 6 were used to study the cytological effect of the fungicides mancozeb and plant guard. The chemical structure of the fungicide mancozeb is [1, 2-ethanediylbis (carbamodithioato) 2] manganese. On the other hand, the biological fungicide plant guard contains the spores of fungus *Trichoderma harzianum*. It contains $30x10^9$ living cells of *Trichoderma harzianum* per liter. *Trichoderma harzianum* is widely distributed member of the soil microflora.

Bulbs *Allium cepa* were germinated in tap water. Actively growing roots were treated with different concentrations for 3, 6, 24 and 48 hrs. Control roots were simultaneously immersed in tap water. After treatments roots were detached, washed and fixed in ethanol: glacial acetic acid (3:1) for 24 hours. Cytological preparations were carried out using Feulgen squash technique (Darlington and La-Cour, 1976). Three replicate were prepared for each treatment and control. All cytological data obtained from the different treatments were statistically analyzed using t-test.

The amount of DNA in the nucleus, DNA ploidy level and the fraction of cells undergoing different phases of cell cycle were calculated from at least 400 cells sampled for each treatment. These include cells with DNA amount less than 2C value, cells with 2C DNA (G_o/G_1), cells with 3C-4C DNA (S phase), cells with 4C DNA (G_2-phase), cells with DNA amount more than the 4C value. These cytometric determinations were made using Leica Qwin 500 Image Analyzer provided with the DNA cytometry software.

Biochemical studies

Characterization of protein profiles was carried out using one dimensional sodium dodecyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). Seeds of *Allium cepa* plants previously their parents were treated with the fungicides mancozeb and plant guard for 24 hours were milled. For protein extraction, 0.2 ml of sample buffer (0.2 M Tris-HCl pH 6.8 containing 2% sodium dodecyl sulfate) was added to 0.02 g of seed meal and stored overnight at 4°C.

Centrifugation was performed at 9000 rpm for 6 minutes, then 60 µl supernatant for each concentration were denatured by heating at 90°C for 3 minutes and loaded in12.5% acrylamide slab gel containing 10% SDS. Run power was 15 mA for about 0.5 h then, raised to 25mA for 6-7 hours.

RESULTS AND DISCUSSION

The cytological effects of the chemical fungicide mancozeb on the *Allium cepa* root tip cells are shown in Table (1). The various treatments with mancozeb have a marked reducing effect on mitotic index values as compared with the control. The mitotic index reflects the frequency of cell division and is regarded as an important parameter in evaluating the rate of root growth. The mitotic index values were progressively decreased as the concentration of the fungicide and the period of treatment increased. The mitotic index reached a minimum value of 1.60% and 2.54% after 48 hours treatments with the concentrations 1.25 and 0.625 gm/L of mancozeb respectively. The reduction of mitotic activity seems to be a common effect of most synthetic pesticides tested for their action on mitosis (Cvikrova *et al*., 2003; Moreno, 2007; Renata-Kontek *et al*., 2007; Singh, 2007).

Reduction in mitotic activity may be due to blocking of mitotic cell cycle during interphase (Mohands and Grant, 1972), inhibition of nuclear-proteins synthesis essential for normal mitotic sequence (Kim and Bendixen, 1987), suppressing of DNA synthesis (Mohanty *et al*., 2004) or change in the relative duration of the mitotic stages (Chauhen and Gupta, 2005).

Cell cycle progression is controlled by checkpoints that mediate the entry into S-phase and mitosis. The progression through these checkpoints is catalyzed by a group of cyclin-dependant kinases (CDKs). The cyclin-dependant kinase, whose activity depends on the association with different classes of cyclins, regulates the entry of plant cells from G_1 into s-phase or from G2 into mitosis (Glab *et al.,* 1994; Binarova, *et al.*, 1998; Marwedel *et al.*, 2002; Harting and Beck, 2006). The association between the inhibitory effect of the fungicide mancozeb with its action on the parameters of the cell cycle it can be concluded that, the reduction in mitotic activity, may be due to arrest of mitotic cycle at the G_2 phase and/or the prolonged duration of S-phase but not to inhibiting the DNA synthesis (Table 3).

The effect of the fungicide mancozeb on the fraction of cell cycle phases as related to the amount of DNA in the nuclei showed that there is a decrease in the proportion of cells with the 2C value as they compared with the control value (Table 3). The proportion of cells with the 2C Value was reduced from 45.63 ±0.29 in the control roots to 30.18 ±0.27 in root cells treated with 1.25 gm/L. On the other hand, there is an increase in the fraction of cells in the S-phase and G_2 phase. The proportion of cells in the S-phase and G_2 phase increased from 27.18 ± 0.27 and 5.95 ± 0.33 in the control to 41.74 ± 0.30 and 16.50 ± 0.35, respec-

tively in roots treated with the highest concentration (2.5 gm/L). The most evident effect appears to be the accumulation of cells in the S-phase and G_2-phase associated with reduction in proportion of cell in G_1 phase. These results indicate that the fungicide mancozeb act as an inhibitor of cell cycle at the G_2 transition point. This result is in agreement with that obtained by Singh (2007) who found that S-phase of cell cycle is more sensitive in comparison to other phases after treatment roots of *Hordeum vulgare* with the fungicide mancozeb. Support for this result is the explanation obtained by Polit *et al.* (2003) who showed that cycline-dependant kinase regulate cell cycle progression and treatment the root meristems of *Vicia faba* with indole 3-acetic acid (IAA), the cytokinin benzyl-6 amino purine (BAP) or a mixture of IAA+BAP, increased the number of G_2 cells, producing a characteristic profiles of nuclear DNA content. Also the action of mancozeb fungicide resembles that of cadmium when applied to *Pisum sativum* roots (Anna-Fusconi *et al.*, 2006). The authors observed that cadmium increased the percentage of 4C nuclei (G_2-phase) and decreasing that of 2C nuclei in addition to a number of mitotic aberrations mainly consisting of sticky metaphase and anaphase bridges.

On the other hand, the results obtained after treatment of the root tips of *Allium cepa* with the biofungicide plant guard for 4, 6, 24 and 48 hours showed a decrease in the mitotic activity which slightly increased with the increase of concentrations but there is no clear decrease in mitotic index at long treatments (Table 2). The statistical analysis of the data reveals that all treatments for 4 and 6 hours had no significant effect on MI. The cytophotometric analysis showed that there is slightly increase in cells at G_1 phase with little change in the proportion of cells at S- phase and G_2 phase as compared with the control (Table 3). The proportion of cells with 2C was increase from 45.63 ± 0.29 in the control to 51.46 ± 0.29 in cells treated with the lowest concentration applied (0.312 ml/L). The fraction of cells in the S-phase fluctuated slightly around the control. The proportion of cells treated with the lowest concentration (0.312 ml/L) recorded 29.67 ± 0.30 as compared with the control, which was recorded 27.18 ± 0.27. The proportion of G_2 phase cells was slightly reduced from a control value of 5.95 ± 0.33 to 4.0 ± 0.21 in root treated with the highest concentration (2.5 ml/L). The proportion of cells with DNA amount less than the 2C value or more than the 4C value generally increased with the increase of concentration.

These results indicate that the reduction in mitotic activity may simply be due to the arrest of mitotic cycle at the G_1 phase or inhibition of nuclear protein synthesis required for the progress of cell cycle. Support for this explanation is the results obtained by Doyle and Bradner (1980) who showed that trichothecine produced by the fungus *Trichoderma* are

potent protein synthesis inhibitors in eukaryotic system due to their binding to 60 S ribosomal subunit and their inhibition of the peptidyl transfrease activity.

In addition to the mitodepressive effect, a number of chromosomal abnormalities covering all stages of mitosis were recorded after treatment with both pesticides mancozeb and plant guard (Tables 1 and 2). The total percentage of these abnormalities increased gradually with the increase of concentrations and the period of treatments. It reached a maximum value of 97.40% and 53.96% after treatment with 1.25 gm/L of mancozeb and plant guard for 48h, respectively. The data reveals that all treatments with mancozeb for different periods had significant or highly significant effect on the percentage of mitotic abnormalities as compared with the control while, treatments with biofungicide plant guard induced significant increase in the frequency of abnormal mitosis at high concentrations and no significant effect at low concentrations. The values of the induced abnormalities after treatment with the biofungicide plant guard were generally low as compared with that scored after treatments with the chemical fungicide mancozeb. This may indicate that, at the cytological level, the chemical pesticides mancozeb is more effective in inducing chromosomal abnormalities in addition to their mitostatic effect as compared with the biofungicide plant guard.

The main effect of the two fungicides used was found on metaphase and ana-telophase stages. At metaphase stages chromosome stickiness, C-metaphase and disturbed metaphase were the most common abnormalities observed. The abnormal spindle formation and the production of this type of abnormalities may be due to the effect of these fungicides on the cyclin-dependant kinases activity. Binarova *et al.* (1998) showed that treatment of *Vicia faba* root tip cells with specific inhibitors to cyclin - dependent kinases leads to abnormal spindle formation in addition, the transcript levels of A and B-type cyclin declined indicating the role of this enzyme in regulating some steps leading to a bipolar spindle structure.

At anaphase and telophase stages, the most common type of abnormality was the formation of bridges. In this study, bridges may be due to stickiness or to breakage and reunion. A considerable number of chromosome breaks were observed in *Allium cepa* roots treated with the synthetic pesticides mancozeb and the biofungicide plant guard. The production of these abnormalities is a true clastogenic effect (Ma, 1982; Andersson and Kihlman, 1992). Laggard chromosomes, tri-multipolar cells, and disturbed configuration were recorded at metaphase and ana-telophase stages. At interphase stage, a few micronuclei and multinucleate cells were observed. The aberrations such as lagging chromosomes, fragments, and micronuclei may lead to an unequal distribution of the genetic

material in the divided daughter cells (Gavrila *et al.*, 1994). This is manifested by scoring of fraction of cells with DNA amount less than the 2C value or more than the 4C value following treatments the roots with the fungicides mancozeb and plant guard (Table 3).

The pesticides used in this study, induced changes in the electrophoretic profiles of seed proteins of *Allium cepa* as compared with the control. The observed changes include alteration in band intensities, appearance of new bands and disappearance of other bands (Table 4 and Fig. 1)**.** The total number of the protein bands were recorded 15 bands, five of which with molecular weights of 70, 48, 46, 34 and 30 KD are common bands to the control and treated roots. The most visible changes in SDS-PAGE patterns were the appearance of new bands with molecular weights of 32, 20 and 12 KD in the treated roots with mancozeb and the band with a molecular weight of 20 KD in roots treated with the biological fungicide plant guard. Two protein bands with molecular weight of 64 and 44 KD were disappeared after treatment with mancozeb while no band was disappeared after treatment with plant guard. An over accumulation was also observed for the protein bands with a molecular weight of 48, 46, 34 and 30 KD. The changes in protein banding patterns have been attributed to the occurrence of either gene mutations or induction of cytological aberrations. The absence of some bands might be due to the deletion of their corresponding genes.

From all the above mentioned results it may be concluded, that the genotoxicity of the synthetic fungicide mancozeb, as indicated by their capacity to produce chromosomal aberrations, was confirmed by their effect on cell cycle phases as well as protein banding pattern and it is more dangerous than of the biological fungicides plant guard. The biological pesticides have antifungal properties and low toxicity. The result of the present investigation, point out the importance of taking proper measure in order to avoid contamination with these pollutants. The toxic effect of the synthetic fungicides has created a demand for new environmentally safe fungicides.

SUMMARY

Biological control agents for plant disease are currently being examined as alternative to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts. In this study root tips of *Allium cepa* were treated with different concentrations ranging from 2.5 to 0.312 gm/L with the synthetic fungicide mancozeb or the biological fungicide plant guard for 3, 6, 24 and 48 hours. The effect of both fungicides on mitotic activity, induction of mitotic abnormalities, percentage of different parameters of the cell cycle and change in seed protein banding patterns has been investigated. At the cytological level both fungicides caused reduction in mitotic index and induced a number of chromosomal abnormalities. The various treatments with mancozeb have a marked

reducing effect on mitotic index values. On the other hand, all treatments with biofungicide plant guard for 4 and 6 hours had no significant effect on MI. Also, the values of the induced abnormalities after treatments with biological fungicide were generally low as compared with those of the chemical fungicide mancozeb. The association between the inhibitory effect of the fungicide mancozeb with its action on the parameters of the cell cycle it can be concluded that, the reduction in mitotic activity, may be due to arrest of mitotic cycle at the G_2 phase and/or the prolonged duration of S-phase. The cytophotometric analysis after treatment with the biofungicide showed that there is slightly increase in cells at G_1 phase with little change in the proportion of cells at S-phase and G_2 phase as compared with the control. The electrophoretic analysis showed that both fungicides induced changes in the protein banding patterns in seed storage proteins as compared with untreated samples.

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Table (1): Frequencies of different types of metaphase and anaphase-telophase abnormalities, mitotic index and mean percentage of abnormal mitosis after treating *Allium cepa* root tips with different concentrations of mancozeb for different times.

Treat-ment	% of metaphase abnormalities							% anaphase-telophase abnormalities						Mean % of MI±SE	Mean % of abn. mitosis ±SE
	Stick.	CM(2n)	CM(4n)	Star	Dist.	Lagg.	Break	Stick.	Bridge	Lagg.	Dist.	Break	Multi polar		
3 hours															
Cont.	-	-	-	-	0.82	-	-	-	-	-	1.38	-	-	7.14±0.26	0.41±0.18
0.321	-	6.87	-	1.53		-	-	-	2.11	-	1.20	1.62	-	5.93±0.09	4.08±0.39
0.625	3.43	4.43	-	1.49	1.99	-	-	10.73	9.25	-	16.82	2.10	0.90	5.47±0.32	17.80±0.19
1.250	13.22	41.28	1.15	0.96	1.15	-	-	33.90	16.04	3.07	8.63	-	5.33	3.85±0.43	53.44±0.22
2.500	33.82	25.65	7.87	2.52	2.20	-	-	39.29	19.98	0.93	0.46	-	5.57	3.27±0.11	65.90±0.23
6 hours															
Cont.	-	-	-	-	1.46	-	-	-	-	-	1.04	-	-	7.42±0.20	0.66±0.02
0.321	-	10.62	-	-	13.09	0.46	1.14	-	2.09	-	2.30	1.20	-	5.67±0.32	9.78±0.19
0.625	3.14	10.50	-	2.72	8.01	1.20	1.60	9.74	8.93	-	10.36	1.21	1.74	5.04±0.27	24.51±0.30
1.250	41.29	14.64	0.16	3.12	7.34	0.16	0.16	48.55	20.02	-	0.29	2.01	2.60	3.82±0.20	63.78±0.16
2.500	46.45	8.50	0.62	6.51	-	-	-	51.33	22.58	-	-	-	0.89	3.41±0.34	67.29±0.07
24 hours															
Cont.	-	-	-	-	1.38	-	-	-	-	-	0.49	-	-	7.07±0.34	0.43±0.13
0.156	-	33.24	-	8.11	13.38	-	-	4.07	23.60	-	18.91	1.02	6.33	5.31±0.19	32.09±0.17
0.321	11.39	24.15	1.59	8.81	12.42	-	-	17.95	24.29	-	16.00	2.92	7.68	4.71±0.18	52.88±0.22
0.625	38.03	23.94	1.41	10.32	20.19	-	-	48.97	21.98	-	5.98	1.10	14.28	4.07±0.25	88.75±0.18
1.250	58.25	16.19	2.06	8.76	9.59	-	-	71.80	19.23	-	-	-	2.55	3.48±0.05	91.78±0.25
48 hours															
Cont.	-	-	-	-	0.62	-	0.62	-	-	-	1.81	-	-	7.19±0.38	0.57±0.20
0.156	-	22.26	10.38	5.28	18.68	-	18.68	-	21.27	-	22.31	8.26	4.49	4.44±0.03	47.13±0.20
0.321	13.96	35.59	8.19	0.82	14.92	-	14.92	45.61	26.44	-	10.02	2.21	4.02	4.21±0.06	74.51±0.17
0.625	48.46	30.28	0.84	1.00	10.14	-	10.14	76.62	21.69	-	-	-	-	2.54±0.10	89.51±0.27
1.250	90.54	8.46	0.50	0.50	-	-	-	91.67	8.33	-	-	-	-	1.60±0.03	97.40±0.14

Stickiness = stick. Diploid abnormalities = abn. c-metaphase(2n) = CM(2n) Mitotic index = MI c-metaphase(4n) = CM(4n) Disturbed = Dist. laggard = lagg.

Table (2): Frequencies of different types of metaphase and anaphase –telophase abnormalities, mitotic index and mean percentage of abnormal mitosis after treating *Allium cepa* root tips with different concentrations of plant guard for different times.

Treat-ment	% of metaphase abnormalities							% anaphase-telophase abnormalities						Mean % of MI±SE	Mean % of abn. mitosis ±SE
	Stick	CM(2n)	CM(4n)	Star	Dist.	lagg	Break	Stick.	Bridge	Lagg.	Dist.	Break	Multi polar		
3hour															
Cont.	-	-	-	-	0.94	-	-	-	-	-	1.35	-	-	4.70±0.04	0.61±0.70
0.321	-	1.04	-	-	7.29	-	-	-	-	1.12	1.02	2.03	-	2.99±0.62	4.38±1.04
0.625	-	3.03	-	-	8.98	2.13	2.02	1.75	1.75	1.75	6.33	2.45	-	2.72±0.82	11.87±1.08
1.250	10.06	12.62	1.24	-	-	2.56	1.24	7.27	16.00	4.00	-	1.82	-	2.62±0.06	21.42±1.08
2.500	20.10	8.20	2.83	-	-	-	-	18.87	18.86	-	-	-	-	2.28±0.35	28.28±0.60
6 hours															
Cont.	-	-	-	-	2.48	-	-	-	-	-	1.32	-	-	4.43±0.16	1.40±0.70
0.321	-	-	-	1.98	7.92	0.99	-	-	-	1.03	2.12	1.03	0.97	2.75±0.46	7.25±1.08
0.625	0.91	0.91	-	0.91	10.35	2.34	4.58	1.25	2.55	-	8.02	2.10	-	2.56±0.42	15.51±1.08
1.250	15.23	5.79	0.81	-	7.44	-	2.13	11.34	4.48	-	-	3.58	2.99	2.34±0.08	24.54±0.60
2.500	30.08	-	3.75	-	-	-	-	21.05	8.77	-	-	-	-	2.19±0.12	28.83±0.60
24 hours															
Cont.	-	-	-	-	2.11	-	-	-	-	-	1.65	-	-	4.25±0.2	1.67±0.40
0.156	-	0.87	2.61	1.73	18.27	0.87	-	-	-	2.15	10.75	-	-	2.63±0.24	16.40±0.70
0.321	5.52	15.63	-	1.56	12.43	-	-	-	6.10	1.29	30.41	-	-2.33	2.55±0.4	31.83±1.08*
0.625	19.85	18.32	-	-	14.50	-	-	33.72	3.48	-	-	-	1.25	2.45±0.04*	43.20±1.08**
1.250	32.07	20.99	3.45	-	-	-	-	25.00	27.50	-	-	-	-	2.41±0.12*	57.50±1.08**
48 hours															
Cont	-	-	-	-	3.65	-	-	-	-	-	2.42	-	-	3.97±0.09	1.92±1.47
0.156	-	-	-	1.11	23.81	-	2.22	-	-	3.70	15.06	6.24	-	2.43±0.43	20.92±0.40
0.321	4.13	9.92	-	2.32	24.24	-	6.23	16.55	14.28	2.13	19.05	5.13	4.76	2.13±0.1*	45.14±0.40*
0.625	14.17	18.61	1.60	-	20.40	-	-	19.05	12.28	6.24	14.28	5.29		2.09±0.16**	51.43±0.40**
1.250	30.79	21.73	4.17	-	-	-	-	33.34	33.33	-	-	-		2.03±0.17**	53.96±0.40**

Stickiness = stick. Diploid abnormalities = abn. c-metaphase(2n) = CM(2n) Mitotic index = MI c-metaphase(4n) = CM(4n) Disturbed = Dist. laggard = lagg.

Table (3): Effect of the fungicides mancozeb and plant guard on the cell cycle parameters in root meristematic cells of *Allium cepa* treated for 24 hours.

Treatment gm/L	DNA less than 2C±SD	G1 Phase ±SD 2C	S-phase ± SD	G2 phase ± SD	DNA more than 4C±SD
Control	25.24±0.14	45.63±0.29	27.18±0.27	5.95±0.33	0.00
Mancozeb					
1.25	2.94 ± 0.17	32.03 ± 0.27	41.74 ± 0.30	16.50 ± 0.35	6.79 ± 0.23
0.625	4.29 ± 0.42	30.18 ± 0.27	39.62 ± 0.21	16.48 ± 0.18	9.43 ± 0.32
0.312	5.81 ± 0.19	30.51 ±0.17	35.46 ± 0.31	15.21 ±0.17	10.01 ± 0.14
0.156	57.78 ±0.03	30.76 ± 0.25	34.62 ± 0.27	14.42 ± 0.33	14.42 ± 0.85
Plant guard					
1.25	16.00 ± 0.32	49.00 ± 0..28	26.00 ± 0.28	4.00 ± 0.21	5.00 ± 0.68
0.625	15.89 ±0.12	45.99 ± 0.31	28.04 ± 0.31	2.41 ± 0.23	2.67 ± 0.71
0.312	10.56 ± 0.23	51.40 ± 0.27	28.03 ± 0.24	7.34 ± 0.39	2.67 ± 0.28
0.156	12.08 ± 0.07	51.49 ± 0.29	29.67 ± 0.30	5.82 ± 0.07	0.97 ± 0.10

Table (4): Effect of the chemical fungicide mancozeb and the biofungicide Plant guard on the protein-banding pattern of *Allium cepa* seed separated using SDS - PAGE technique.

Band No.	M wt	Control	Plant guard				Mancozeb			
			Lane P1	Lane P2	Lane P3	Lane P4	Lane M1	Lane M2	Lane M3	Lane M4
1	72	-	-	-	-	-	+	+	+	+
2	70	+	+	+	+	+	+	+	+	+
3	68	-	-	-	-	-	+	+	+	+
4	64	+	+	+	+	+	+	-	-	-
5	48	++	++	++	++	++	++	++	+++	++
6	46	+	++	++	++	++	+	+	++	++
7	44	+	+	+	+	+	+	+	-	+
8	42	-	-	-	-	-	+	+	+	+
9	34	+	++	++	++	++	++	++	++	++
10	32	-	-	-	-	-	+	+	-	-
11	30	++	++	++	++	++	++	++	++	++
12	20	-	+	-	-	-	+	+	+	+
13	14	+	+	+	+	+	+	+	+	+
14	12	-	-	-	-	-	-	-	+	-
15	10	+	+	+	+	+	+	+	+	+
Total		9	10	9	9	9	14	13	12	12

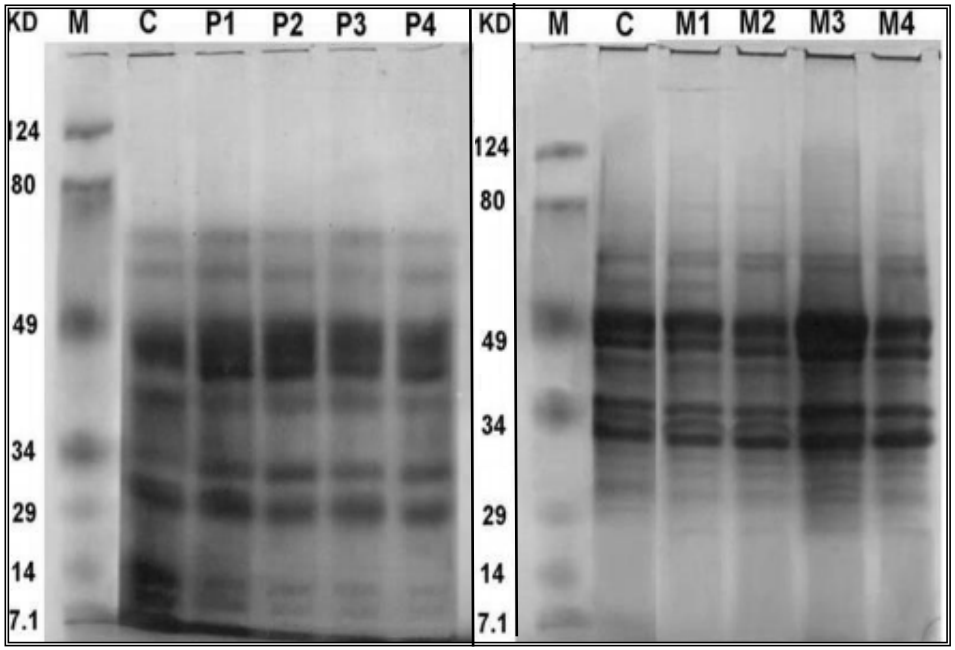

Fig. (1): Electrophotograph produced by SDS-PAGE analysis of protein pattern of *Allium cepa* seed after treatment with different concentrations of plant guard and mancozeb.

M = Marker	C = Control	P = Plant guard	M = Mancozeb
Lane 1:0.312%	Lane 2: 6.25%	Lane 3: 1.25%	Lane 4: 2.50%