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Studies on the Antioxidative stress responses of fungicides carbendazim and mancozeb in seedlings of brassica (*Brassica compestris*L.)

Gurpreet Singh* and Daljit Kaur
Post-Graduate Department of Biotechnology, Lyallpur Khalsa College, GT Road, Jalandhar -144001 Punjab, India
s_gp@rediffmail.com, drgurpreet@lkc.ac.in

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Abstract

The present study is an attempt to understand the effect of different concentrations of fungicides – carbendazim and mancozeb on the various antioxidative enzymes in developing seedling of brassica. Seeds were treated with different conc. (0.01-0.05%) of fungicides for 3 hours and then allowed to grow for 10 days in an incubated chamber. Treatment of fungicides triggered a defence machanism of various antioxidative enzymes viz. ascorbate perosidase, guaiacol peroxidase, catalase and superoxide dismutase and it was observed that the level of catalase and peroxidase decreased at higher conc of fungicides as compared to control samples. Mancozeb treatment, however showed an increase in the conc of catalase activity. An overall increase in the activity of superoxide dismutase was observed in samples treated with carbendazim, mancozeb individually as well as in combination thus suggesting that plant induces various antioxidative enzymes as protective defence mechanism against fungicide stress. These results indicate that the application of fungicide singaly or in combination in brassica seedlingsis deleterious at higher dosages and should be used judiciously in current agriculture.

Keywords: Carbendazim, Mancozeb, Brassica, Antioxidant enzymes, Fungicides.

Introduction

Fungicides are among the class of pesticides, which are either chemicals or biological agents that are known to prevent the growth of fungi on plants. Application of various systemic or broad spectrum fungicides are reported to affect cause growth reduction and disruption in development ultimately leading to low plant growth and yield[1,2]. Recent studies suggest that extensive use of fungicides at higher conc. interfere with various metabolic pathways such as carbon and nitrogen metabolism, photosynthetic activity and chlorophyll biosynthesis and induces a stress response mechanism to counter thedamages caused by the application of these fungicides[3,4].

Carbendazim is a broad spectrum systemic fungicide belonging to which belongs to benzimidazole chemical group and Mancozeb is a contact fungicide of chemical group dithiocarbamate that react with the protein SH groups. It has been reported that these fungicides effects the growth and plant shoot length and antioxidant enzyme assay[5]. Fungicides are known to induce the chain of antioxidative enzymes such as peroxidases, catalases and superoxide desmutase which counters the plant from deleterioius effect of fungicdes and acts a defense mechansim against such stresses[6].

Brassicacompestris botanically belongs to the family *Brassicaceae*and the genus-*Brassica*includes widely used agricultural and horticultural crops. It is the third important oilseed crop in the world after soybean (*Glycine max*) and palm (*Elaeisguineensis* Jacq.) oil and India share nearly27.8% in the country's economy from this crop[7]. This study was aimed to investigate the effect of different fungicides on the growth parameters, biochemical and antioxidative parameters on the developing seedling of *Brassicacompestris*.

Materials and Methods

The seed samples of *Brassica campestris*L. was collected from local market of Jalandhar and adjoining areas. Dry seeds were washed with thoroughly first in running water followed by dipping in distilled water for 10 minutes. The pre-soaked seeds were incubated separately with fungicide carbendazim and mancozeb with 0.01- 0.05% concentrations for 3hrs. Seeds were dipped in the distilled water at the same time to serve as control. The control and stressed samples were placed on the double layered filter papers (Whatmann paper No. 3) wetted with distilled water in Petri dishes. Incubation was given at 25°C for 10 days with regular supply of water. Plants were harvested after 10 days and stored at -20°C for further analysis.

Ascrobate peroxidase (APX) activity: The ascorbate peroxidase enzyme activity was assayed using the mixture consisted of 200µl of enzyme extract, 1500µl of sodium phosphate buffer (50mM), 400µl of 0.5M ascorbate solution. 200µl of H_2O_2 with the final addition of 700µl of the distilled water as per the protocol[8]. The ascorbate oxidation was measured by recording the absorbance at 290nm at the time of H_2O_2 addition and 5 min later. The APX activity was determined by dividing the difference in absorbance by molar extinction coefficient ($2.8mM^{-1}cm$).

Guaiacol peroxidase (GPX) activity: The guaiacol peroxidase activity was measured according to Egley *et al.*[9] Reaction mixture consist of 200µl of enzyme extract, 200µl of the H_2O_2 , 400 µl of guaiacol, 1.5ml of sodium phosphate buffer and 700µl of distilled water. The absorbance was recorded at 420nm at the time of H_2O_2 addition and 2 min later. GPX activity was obtained as follows

Activity = $A_{420\ (2\ min)}$-$A_{420\ (0\ min)}$/ 26.6.

Catalase (CAT) activity: Assay mixture for catalase activity consisted of 150µl of the enzyme extract, 500µl of the sodium phosphate buffer (50 mM), 500 µl of the H_2O_2 and 550 µl of distilled water as per the protocol of Abei and coworkers[10]. The decrease in H_2O_2 was monitored by recording the difference in absorbance at 240nm at the time of H_2O_2 addition and 1min later divided by molar extinction coefficient ($36mM^{-1}cm$).

Superoxide dismutase (SOD) activity: The superoxide dismutase activity was measured according to Roth and Gilbert[11]. Reaction mixture consisted of the 200µl of the enzyme extract, 500µl of the sodium phosphate buffer, 300µl of the pyrogallol in 2 ml final reaction mix. The SOD activity was monitored at 420 nm for 3 min.

Results and Discussion

Brassica plants were exposed to different levels of two fungicides, which inhibited the growth of the plants in term of shoot lengths, shoot and root dry and fresh weights, lipid peroxidation and antioxidant enzymes.

A gradual decrease in the shoot length was observed with the increase in the concentration of fungicides (Table-1). Exposure to 0.05% conc. of fungicide resulted a drastic decrease in the shoot length as compare to the control plants. With the increase in the concentration of carbendazim fungicide shoot length of brassica reduced gradually. Exposure of carbendazim fungicide effected the shoot length of brassica by 69% and 61% in 0.04% and 0.05% conc. of fungicides. In mancozeb exposed plants shoot length was decreased by 79% in 0.05% conc. While combined effect of both the fungicides decreased the shoot length by 78% at 0.03-0.05% concentrations.

Data indicates that as carbendazim conc. was increased the APX activity was increased by 43% and 69 % as compared to the control (Figure-1). Increased conc. of mancozeb fungicide had resulted increase in APX activity and at 0.02% conc . of fungicide APX activity was increased by 244%. While at 0.03% exposure value was decreased by 33%. Combined effect of both the fungicides resulted decrease in APX activity at higher conc. by 45% , but maximum was obtained at 0.01% (195%) exposure.

Incraesed conc of carbendazim stress to brassica seedlings resulted in incresed activity of guaiacol peroxidase (Figure-2). Carbendazim fungicide had resulted a overall increase in GPX activity at various conc. of fungicide. With mancozeb fungicide maximum GPX activity was 126% at 0.03% conc. while in other conc. GPX activity was reduced by 46% (0.01%) and 85% (0.05%). Combined treatments of carbendazim and mancozeb fungicide resulted increase in GPX activity by 164% and 201% in 0.02% and 0.03% conc. of fungicide. Minimum value was obtained at 0.01% conc. of fungicide (13%).

It was observed that maximum catalytic activity was observed at 0.04% and least was obtined at 0.01% compare to the control. Catalytic activity decreased to 40%, 80% at 0.01% and 0.05% conc of fungicide (Figure-3). Maximum catalase activity was recorded at 0.04% (240%) as comaperd with the control sample. Exposure of mancozeb at higher conc from 0.02-0.05% resulted in an incresae in the catalase activity by 184%, 146%,176% and 346% respectively. Minimum catalytic activity was obtained at 0.01% (53%) conc. of fungicide. Combined treatment of carbendazim and mancozeb fungicide decreased the catalase activity by 63% respectively.

Table-1
Average seedling length of brassica plants exposed to variousconcof fungicide stresses

Sample – Seedling	Effect of fungicides		
	Carbendazim	Mencozeb	Carbendazim + Mencozeb
Control	3.18 ± 0.21	3.74 ± 0.31	3.20 ± 0.26
0.01%	3.06 ± 0.23	4.48 ± 0.41	2.24 ± 0.19
0.02%	2.84 ± 0.56	3.92 ± 0.35	2.28 ± 0.24
0.03%	2.76 ± 0.40	3.72 ± 0.24	2.24 ± 0.26
0.04%	2.22 ± 0.32	3.14 ± 0.29	2.20 ± 0.18
0.05%	1.94 ± 0.51	2.96 ± 0.37	2.04 ± 0.26

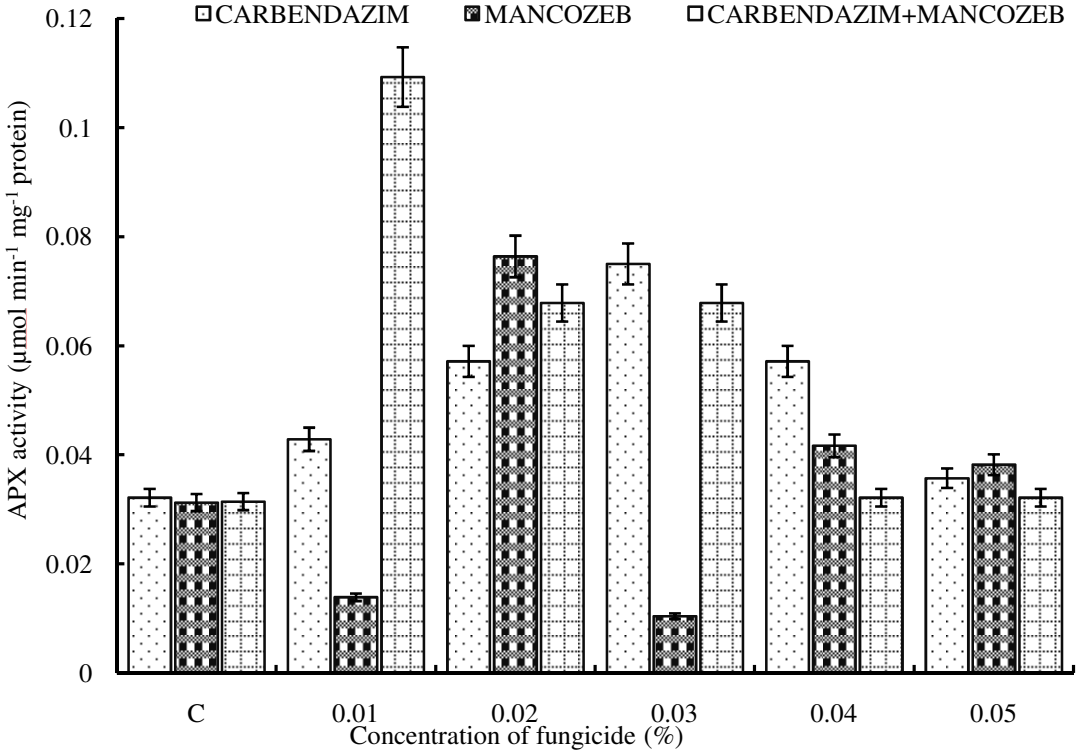

Figure-1

Effect of different fungicides on ascorbate peroxidase content in seedlings of brassica under different conc. (Error bars indicate mean ± SD (n=3) with P value ≤ 0.005)

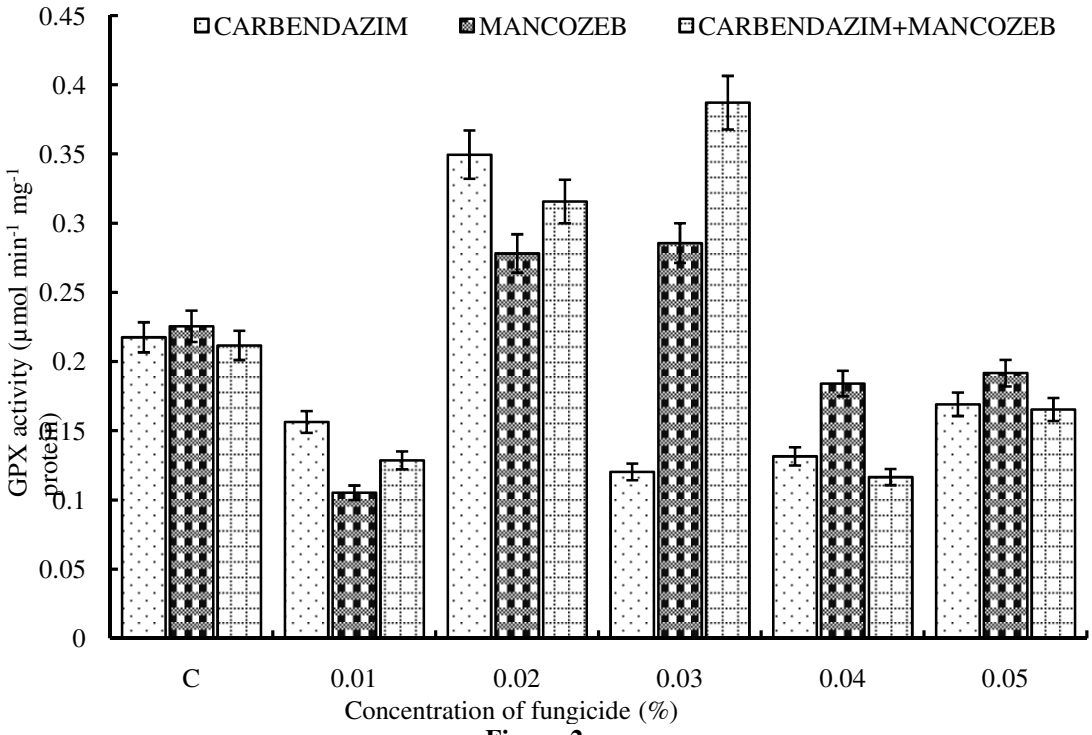

Figure-2

Changes observed in ascorbate peroxidase content in seedlings of brassica under different conc. of fungicides. (Error bars indicate mean ± SD (n=3) with P value ≤ 0.005)

Exposure of carbendazim fungicide at 0.02% and 0.04% varied the SOD activity by 78% and 68% (Figure-4). Maximum SOD activity was obtained at 0.03% conc. of fungicide which was 113%. An increase of 103-148% in the SOD activity was observed in case of Mancozeb stresses brassica seedlings subjected to 0.01-0.05% conc. respectively. In combined treatment maximum SOD activity was increased gradually and maximum was obtained at 0.05% conc. which was 223% while minimum SOD activity was 90% in 0.01% conc. of fungicide.

Pre and post treatment with fungicides such as periodical sprays constitute one of the most potent method to crub the diesases associated with fungal infection. However, besides the beneficial effects, fungicides impairs various physiolocial, biochemical and metabolic pathways. The toxic effect of such fungicide on developing seedlings depends on their concentration,distribution, persistence, metabolismand its active form. Present study is an therefore attempt to examines the toxic effects of two fungicides (carbendazim and mancozeb) on various physiological, biochemical and antioxidative enzyme activities[13].

It has been suggested that the use of systemic fungicides caused significant change in morphology, seedling growth and chlorophyll content in plants[14]. We observed that the application of different fungicides (carbendazim and mancozeb) in brassica seedlings decreased their overall growth pattern viz. seedling length, relative water content etc. with the increased conc. The reduction in growth parameters could be due to the interference of higher conc of fungicide in various metabolic processes viz. photosynthetic activity,membrane composition, water utilizisation etcultimately resulting in the reduction of biomass production[5,13].

Peroxidases such as ascorbate peroxidase and guaiacol peroxidaseses are an indespensible component of ascorbate-glutathione pathway, required to scavenge H_2O_2 produced mainly in chloroplast and other cell organelles and to maintain the redox state of the cell[15]. In this study we observed an increase in the APX and GPX activity at different conc of fungicides with applied individually or in combination. peroxidasesconstitutes an important classof antioxidant enzymes in plants that detoxify the formation of hydrogen peroxide ions. APX and GPX is known to reduces H_2O_2 to water by ascorbate and guaiacol as specific electron donor[16]. Catalases is oxidoreductase that converts H_2O_2 to water and molecular oxygen and are reportedas key enzymes to remove of toxic peroxides induced under fungicide stress[6,13]. While in carbendazim and combined fungicide exposed plants there was increase and decrease in the catalase activity at certain concentrations of the fungicides. Studies suggest that CAT levels increase proportionally with increased conc of ungicide concentration in wheat and tomato[17]. SOD initiates detoxification of singlet oxygen by forming H_2O_2 ,which is also toxic and must be eliminated by conversion of H_2Oin subsequent reactions.

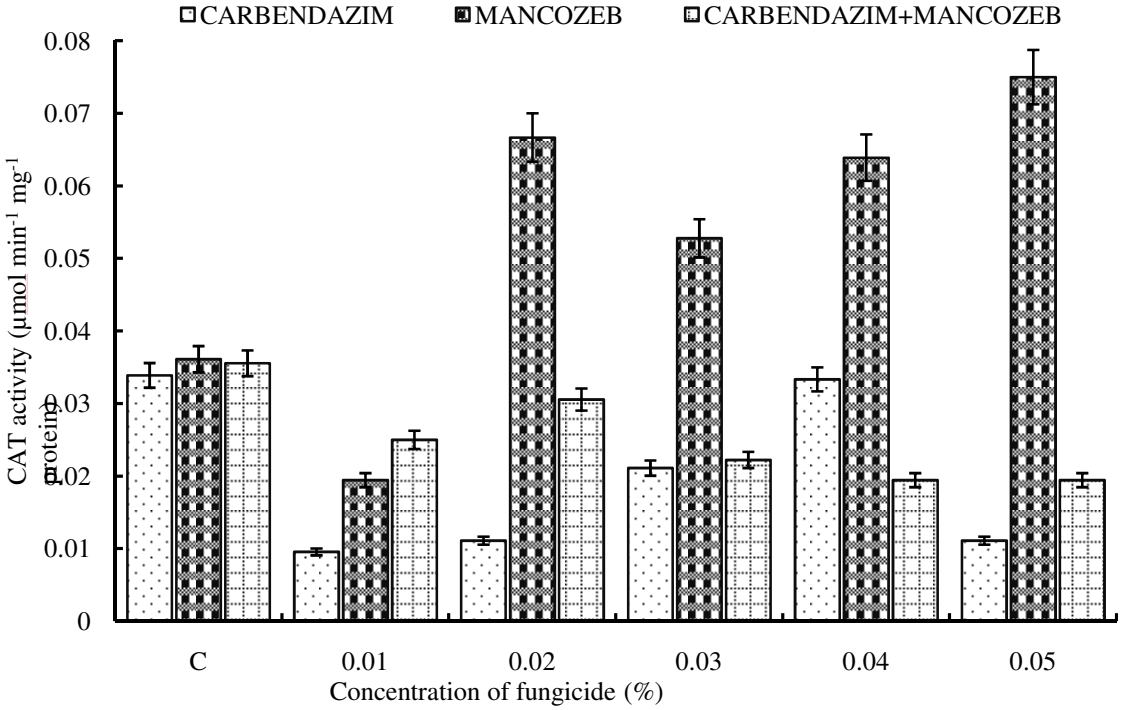

Figure-3
Changes observed in catalase activity in seedlings of brassica under different conc of fungicides. (Error bars indicate mean ± SD (n=3) with P value ≤ 0.005)

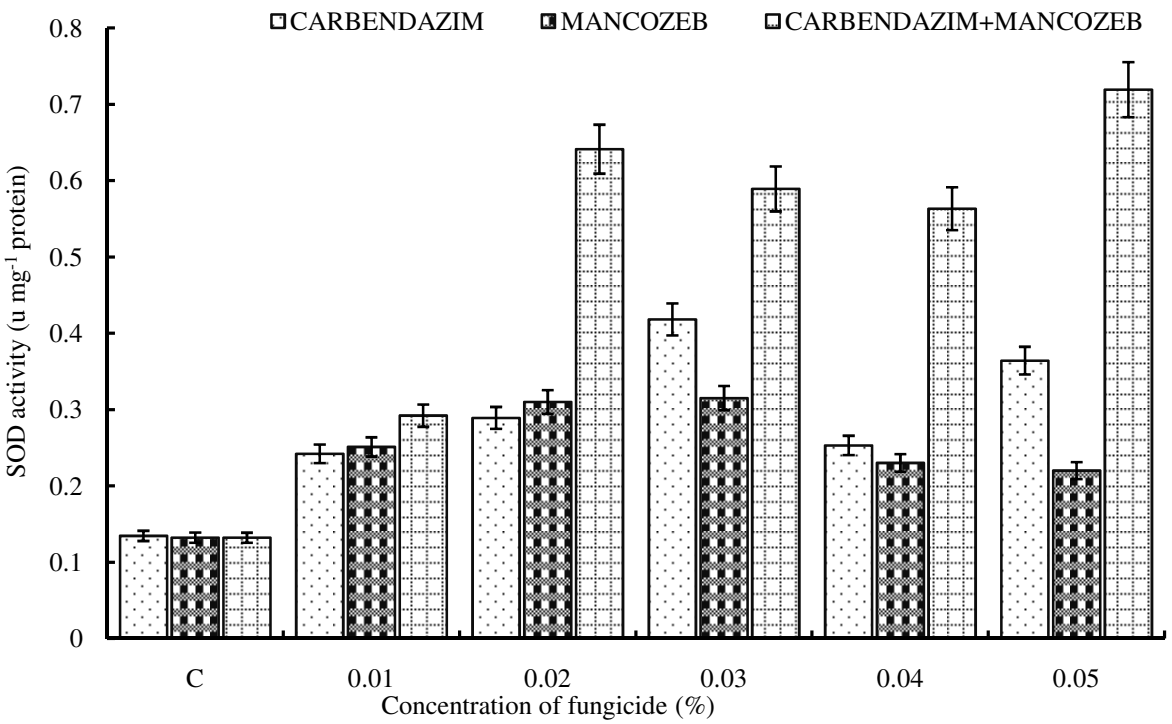

Figure-4
Effect of different fungicides on superoxide dismutase activity in seedlings of brassica under different conc. (Error bars indicate mean ± SD (n=3) with P value ≤ 0.005)

Superoxide dismutases are the enzymes which helps in metabolizing the singlet oxygen $O^{2\bullet-}$which is induced in response to various abiotic stress including pesticides and fungicides and cause extensive damage to cellular metabolic presses and induces a cascade of deleterious reactive oxygen species (ROS). The dismutation of superoxide radicals intoH_2O_2 and oxygen is an important step in protecting the cells and is catalyzed by SOD. Our observation indicates an enhancement in activity of SOD in response to both the types of fungicides.The transient behaviour of fungicides on the antioxident enzymes in developing seelings could be attributed to the increased utilization of these antioxidants to combat the reactive oxygen species generated during the oxidative stress[18], thus corroborating that higher conc of this enzyme acts in defense to resist higher conc. of fungicides in brassica.

Conclusion

We conclude that the treatment of brassica seedlings with the different conc of fungicides affects the overall physiological and antioxidative profiling in brassica seedlings and induces various negative and positive metabolic and biochemical changes.

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