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Effects of Amistar and Dithane M-45, a systemic fungicide, on Growth Parameters and antioxidative enzymes of Maize (*Zea mays* L.).

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Research Article

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ABSTRACT

Fungicides are used to protect the most common Seeds of various fungal diseases. The use of fungicide, whether beneficial or Harmful for the growth of crops is an issue for a long time. So the present study was aimed at evaluation of the effect of Amistar and Dithane M-45fungicides on various growth parameters and antioxidative enzymes of Maize seedlings. The parameters studied were germination percentage, root length, shoot length, vigor index, fresh weight, dry weight and viability percentage. Five different concentrations of fungicide were used, including recommended dose of fungicide. In all the concentrations studied there was an increase in the percentage of germination, root length, and the length of the shoot, and the signal strength indicator, fresh weight and the proportion of feasibility and a decrease in dry weight. In each lesson concentrations there was an increase In the percentage of germination, root length, and the length of the shoot, and the signal strength indicator, fresh weight and the proportion of feasibility and a decrease in dry weight. The results after treatment with Amistar and Dithane M-45 show increased levels of antioxidative enzymes in *Maize* roots. Activities of catalase, Superoxide dismutase, thioredoxin reductase ascorbate-peroxydase and guaiacol-peroxydase increased proportionally and were more meaningful at high concentrations (75 and 100 ppm). It was concluded from this study that there is an increase in the water holding capacity of the seedlings in the presence of fungi in the recommended dose but a higher dose than recommended could prove harmful for the growth of the seedlings.

INTRODUCTION

The extensive use of fungicides, which pose more of carcinogenic risk than other pesticides [2] may lead to undesirable effects on animals and humans biological [21]. Seed treatment with systemic fungicides is the traditional method used to control the infection, which is transmitted by seeds in the intensive production of maize. Seed treatment with the proper formulation affect the initial development of plants which, in turn, will Tatmurahal subsequent growth and development, and finally, the levels of return [10].

Maize is one of the best researched and is characterized by plants, and large amounts of information available on many aspects of the biology of corn and corn is an annual grass growing up to 4 meters in length.

Plants have an advanced defense system against ROS involving enzymatic and non-enzymatic means. Catalase, ascorbate-peroxydase and guaiacol-peroxydases are antioxidant enzymes, which play a capital role in keeping H₂O₂ levels harmless and therefore contribute to protecting plants from ROS damages [13].

Fungicides chosen for the study were Amistar and Dithane M-45. Amistar is a broad spectrum and systemic fungicide produced by Syngenta. It has an active ingredient of 250 g/L azoxystrobin, which possesses a novel biochemical mode of action: it inhibits mitochondrial respiration in fungi [3]. It is active on all four classes of fungi that attack crops. It is registered for use on more than 80 crops in 85 countries worldwide. Today, farmers use Amistar for fungal control in cereals such as Amazon and barley as well as in vines, fruits, vegetables, bananas, rice, soybeans, turf and ornamentals.

Dithane M-45 is a broad spectrum, contact fungicide produced by Dow Agro Science, Inc. Its active ingredient is 800 g/kg Mancozeb that has a multi-site mode of action that affects many enzymes in the fungi [4]. Dithane M-45 is recommended for control of a wide range of diseases. Optimal disease control is achieved when the fungicide is applied in a regularly scheduled, preventative a pray program.

There is very little information available regarding the effect of fungi on legumes and cereals in general and Maize in particular. Therefore, this study undertaken in order to understand the impact of fungi commonly used. Amistar and Dithane M-45 on the growth of maize seedlings.

MATERIAL AND METHODS

Synthetic fungicides

Fungicides chosen for the study were Amistar and Dithane M-45, which are used currently on the pepper farm. The former was obtained from the Syngenta Company and the latter from the L&L Pepper Farm. Fungicides usually are mixed on the farms and applied at the rate of 175-210 g/ha of Amistar (280 g mixed in 900 L water) and 3-4kg/ha of Dithane M-45 (2 kg mixed in 900 L water) (L. Campagnolo, personal communication)

Maize (*Zea mays* L.)

The genus *Zea* belongs to the tribe Andropogoneae in the subfamily Panicoideae in the family Poaceae. There are five species included in the genus *Zea*.

Growth Parameters

The fungicides Amistar and Dithane M-45 were used for the present study. The four different concentrations (25, 50, 75 and 100 ppm) solutions of the systemic fungicides (Amistar and Dithane M-45) were used in the study. Healthy Maize seeds were disinfected at 2.5% sodium hypochlorite for 3 min in ethanol, and then washed using sterilized water. Germination was conducted in germination boxes (Gerbox), and seeds were placed within a double sheet of previously sterilized filter paper. Seeds were incubated in gearbox containing 25, 50, 75 and 100 ppm solution of the systemic fungicides (Amistar and Dithane M-45). Untreated Petri plates served as control. The seeds were germinated under controlled conditions. The seeds were then allowed to germinate for 24 hrs. Germinated seeds were then transferred in gearbox lined with Whatmann filter paper no. 1. At the start of the experiment 3 ml of respective concentration was added to moisten filter paper in each gearbox and every day, 2 ml of respective concentration was added for consecutive 6 days. Three sets in each concentration were maintained along with the control for comparison. On the seventh day, various growth parameters were evaluated as follows:

Germination percentage

Germination percentage was estimated by the below formula given by Rehman et al., [22]:

$$\text{Germination \%} = \frac{\text{no. of seeds germinated}}{\text{total no. of seeds}} \times 100$$

Root and shoot length

Root and shoot length of seedlings were recorded using the standard centimeter scale [14].

Vigor index

Vigor index was calculated using following formula suggested by Abdul Baki and Anderson [1]:

vigor index = germination % × (root length + shoot length) * (* indicate that root length and shoot length should be in cm)

Fresh and dry weight

Four seeds of each treatment were weighed in order to determine the fresh weight and then dried in oven at 80 °C for 24 hours to obtain the dry weight [14]. Fresh weight and dry weight were recorded in gms.

Viability percentage

It was determined using the method given by Lakon [15] in which colorless triphenyl tetrazolium dye was used which turns red when is reduced by respiring embryo i.e. indication of germination.

For extraction of antioxidative enzymes, eight days old root tips were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The extraction was performed as described by Loggin et al. [16]. Enzyme activities in each extract were determined using a diode array spectrophotometer. The assay was conducted in a total volume of 3 ml at 25 °C for 3 min and the results were repeated three times using 15 - 20 root tips.

Catalase (CAT), were assayed according to the method of Cakmac and Horst [8], Superoxide dismutase [20], sorbitol dehydrogenase, thioredoxin reductase, guaiacolperoxydase (GPX), [20], ascorbate-peroxides (APX), [19] and Proteins in each extract were assayed according to the method of Bradford [7].

RESULTS

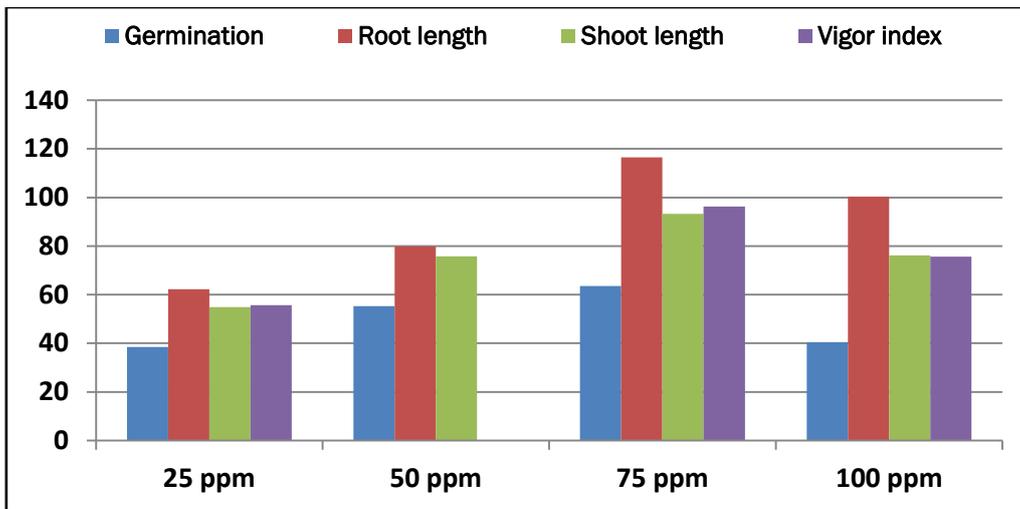
The present results in Table 1. Showed increase in germination percentage, root length; shoot length, vigor index of maize seedlings by effect of different concentration of fungicide. Germination percentage in control was 56.33±3.3 and at 25, 50, 75 and 100, ppm concentration of Amistar fungicide was 78.0±6.1, 87.5±1.8, 92.13±3.4 and 79.16±1.3 respectively. The highest increase in germination percentage was observed at 75 ppm concentration of Amistar fungicide. Germination percentage in control was 56.33±3.3 and at 25, 50, 75 and 100, ppm concentration of Dithane M-45 fungicide was 69.0±3.1, 78.5±2.7, 85.16±1.8 and 91.12±2.4 respectively. The highest increase in germination percentage was observed at 100 ppm concentration of Dithane M-45 fungicide. The present data showed that Root length in untreated maize seedling was observed to be 5.45 ± 1.2 cm. Root length of maize seedlings treated with 25, 50, 75 and 100 ppm of Amistar fungicide was found to be 8.84 ± 2.5, 9.8 ± 4.2, 11.8 ± 1.43 and 10.6 ± 0.58 cm respectively. Maximum root length was found at 75ppm concentration of Amistar fungicide. Root length of maize seedlings treated with 25, 50, 75 and 100 ppm of Dithane M-45 fungicide was found to be 7.46 ± 1.8, 8.94 ± 1.1, 10.2 ± 2.6 and 11.2 ± 1.12 cm respectively. Maximum root length was found at 100 ppm concentration of Dithane M-45 fungicide. The present data also, showed that the short length in control seedlings was 6.2±1.10 cm and at 25, 50, 75 and 100 ppm concentration of Amistar fungicide was found to be 8.12 ± 0.64, 9.21 ± 0.52, 10.13 ± 0.73 and 9.23 ± 0.81 cm respectively. The highest increase was found at 75 mg/l concentration of Amistar fungicide. While the short length of maize seedlings treated with 25, 50, 75 and 100 ppm of Dithane M-45 fungicide was found to be 6.88 ± 1.8, 7.8 ± 2.6, 8.8 ± 2.5 and 10.4 ± 0.78 cm respectively. The short length was increased with increase in concentration of fungicide. The present data showed that the Vigor index of maize seedlings treated with 25, 50, 75 and 100 ppm was found to be 1433.2 ± 221.2, 1697±82.2, 1806.4 ± 240.7 and 1616.2 ± 23.1 respectively when compared with control (Vigor index of maize seedling in control was (920.1±120). While Vigor index of maize seedlings treated with 25, 50, 75 and 100 ppm of Dithane M-45 fungicide was found to be 1311.2 ± 111.4, 1421 ± 54.2, 1588.4 ± 256.2 and 1686.4 ± 44.2 respectively.

The present results in the table 2. Showed that increase in fresh weight, dry weight and viability % of maize seedlings by treated with fungicide.

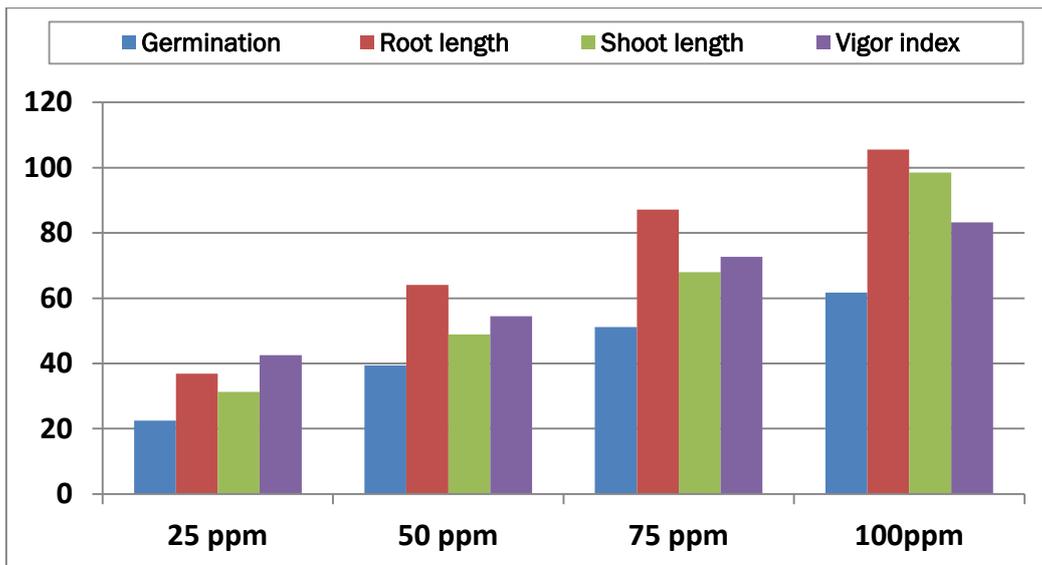
The present data showed that Fresh weight of maize seeds treated with Amistar and Dithane M-45 fungicide increased when compared with Control. Fresh weight of maize seeds treated with Amistar at 25, 50, 75 and 100 ppm was found to be 0.84 ± 0.31, 1.18 ± 0.18, 1.26 ± 0.26 and 1.31± 0.41 and 1.31 ± 0.32 gms respectively. While, dry weight of maize seeds treated with Amistar and Dithane M-45 fungicide then decreased when compared with Control. Dry weight of maize seeds treated with Amistar at

at 25, 50, 75 and 100 ppm was found to be 0.11 ± 0.018 , 0.10 ± 0.045 , 0.10 ± 0.22 and 0.09 ± 0.16 gms respectively.

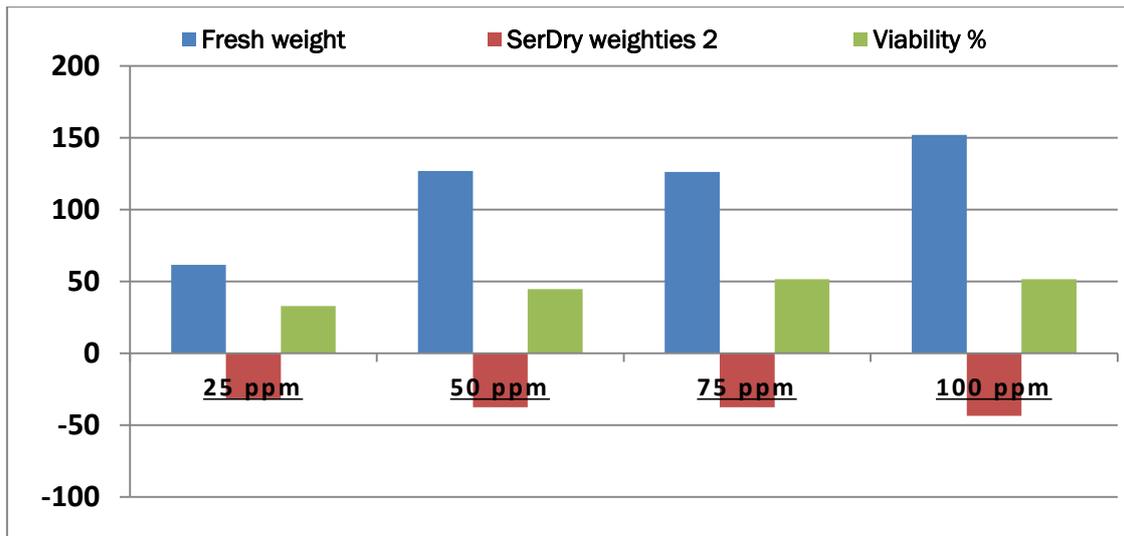
The present data also, showed Viability percentage, of maize seedlings at 25, 50, 75 and 100 ppm of Amistar fungicide was found to be 72.12 ± 3.3 , 78.5 ± 6.7 , 82.22 ± 4.2 and 88.6 ± 6.1 when compared with control (54.22 ± 4.2), respectively. While of Viability percentage, of maize seedlings treated with 25, 50, 75 and 100 ppm of Dithane M-45 fungicide was found to be 68.16 ± 2.8 , 72.8 ± 4.4 , 80.22 ± 3.2 and 81.55 ± 3.5 , respectively. The present results in the table 1 showed that the effect of Amistar and Dithane M-45 fungicide on some antioxidant enzymes CAT, SOD, TrxR, GPX and APX. The data obtained indicated that there was a significant increasing in CAT, SOD, TrxR, GPX and APX enzyme activities as compared to the control. CAT, SOD and TrxR content increased in roots with increase concentrations of Amistar and Dithane M-45 fungicides which reach its maximum at 100 ppm (% and %, respectively). The present data showed that Amistar and Dithane M-45 fungicides induced an increase guaiacolperoxydase (GPX) and ascorbateperoxydase (APX) content in maize root, Which reach its maximum at 75 and 100 ppm (about 80% and 140%, respectively).



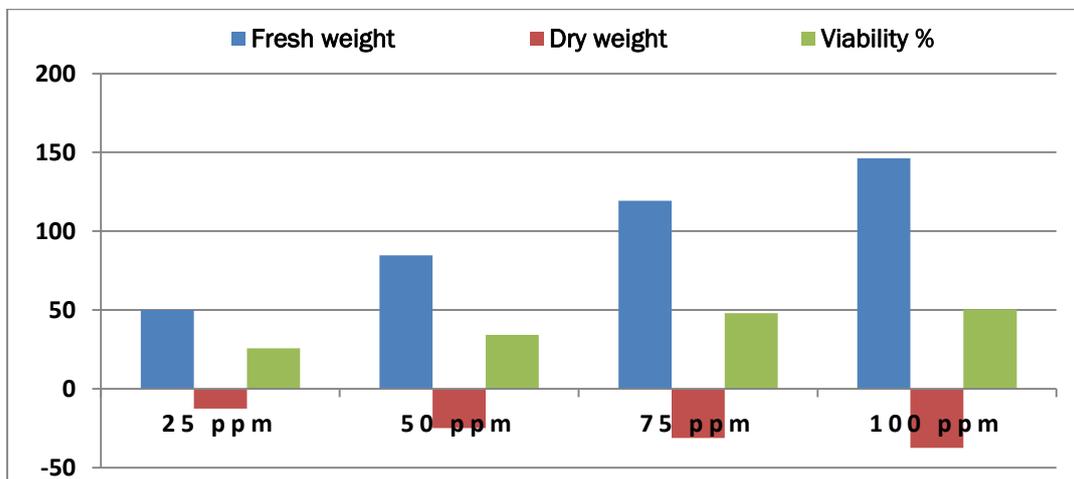
Concentrations of Amistar fungicides
Percentage change of germination, root length, shoot length and vigor index of maize seedlings after treated with concentrations of Amistar fungicide.



Concentrations of Dithane M-45 fungicides
Percentage change of germination, root length, shoot length and vigor index of maize seedlings after treated with concentrations of Dithane M-45 fungicide.



Effect of Dithane M-45 fungicide on Fresh weight, Dry weight and Viability of length, shoot length and vigor index of Maize seedlings.



Effect of Dithane M-45 fungicide on Fresh weight, Dry weight and Viability of length, shoot length and vigor index of Maize seedlings.

Table 1: Effect of Amistar fungicide on antioxidative enzymes activity

Conc. Of fungicide in mg/l	catalase (CAT)	Superoxide dismutase	guaiacol-peroxydase (GPX)	ascorbate-peroxidase (APX)
Control	0.18	0.056±0.01 ^a	0.270±0.03	0.64 ± 1.4
25	0.24 ± 0.21	0.064±0.023 ^b	0.25±0.32 ^b	0.68±1.3
50	0.26 ± 0.12	0.084±0.022 ^a	0.36±0.12 ^c	1.2±1.1
75	0.38 ± 0.14	0.09±0.019 ^h	0.67±35.1 ^f	1.6±1.8
100	0.48± 0.26	0.18±0.41 ^g	0.89±1.4 ^d	1.8±1.2

Table 2: Effect of Dithane M-45 fungicide. On antioxidative enzymes activity

Conc. of Dithane M-45 fungicide in mg/l	catalase (CAT)	Superoxide dismutase	thioredoxin reductase	ascorbate-peroxidase (APX)
Control	0.18±0.2	0.051±0.06 ^a	0.25±0.13	0.51 ± 1.3
25 ppm	0.22±0.6	0.072±1.3	0.31± 0.22	0.81±2.5
50 ppm	0.25±0.8	0.08±1.2	0.41± 0.37	0.84±2.1
75 ppm	0.36±0.45	1.08±1.1	0.46± 0.34	0.92±2.1
100 ppm	0.42±1.2	1.21±1.2	0.54± 0.39	1.2±2.8

DISCUSSION

The present result indicated that the concentration of Amistar and Dithane M-45 fungicides highly effects on growth parameters of maize seedlings and growth parameters of maize seedlings increased with increase in concentration of fungicide. This result is supported by Horii *et al.*, [9,7]. They found that seed germination was stimulated by thiamethoxam in soybean, pea and corn. This results are contrary to Marini *et al.*, [17] who reported that fungicide produces negative interference in germination of seeds. The present data indicated that root length and shoot length was increased with increasing concentrations of Amistar and Dithane M-45 fungicides. This agree with Sarkar and Saxena [24] and Bensoltane *et al.*, [6]. The present study results were contrary to Windham & Windham [26] who have indicated that systemic fungicides which are based on sterol biosynthesis inhibitor are closely related to plant growth regulators the use of which at higher than labeled rates shorten the internodes which may lead to slow shoot growth. Also, The present data indicated that the increase in vigor index was significant at all the concentration of Amistar and Dithane M-45 fungicides as compared to control. These findings are supporting by Doyle *et al.*, (2001) who proved that seedlings treated with thiamethoxam had a particular advantage of improved seedling vigor.

The present data indicated that increase in fresh weight of maize seedlings treated with increasing concentrations of Amistar and Dithane M-45 fungicides. These results agree with the findings of Avinash and Hoshmani [5] that fresh weight of the leaves of sorghum seeds treated with carbendazim increase with increase in concentration of fungicide. The results were also similar to the effect of tricyclazole on Maize seeds [5]. The data showed that the dry weight gets decreased under the effect of fungicide which may be due to increase in water retention capacity of seedlings. The results were contrary to Minamor [18] who reported no difference in dry weight of fungicide treated and untreated cocoa seedlings. According to the present results Viability percentage was increased with increase in concentration of fungicide.

The present study indicated that CAT, SOD and TrxR content increased in roots with increase concentrations of Amistar and Dithane M-45 fungicides. The absorption of Amistar and Dithane M-45 active ingredients by seeds. Later germination outbreak involving penetration in various tissue cells root. The absorption of Amistar and Dithane M-45 active ingredients by seeds Later germination outbreak involving penetration in various tissue cells root. In response to fungicides toxic effects, root cells mobilize a set of detoxifying mechanism which are largely dependant on ATP in order to maintain a possible normal growth rate [11]. Amistar and Dithane M-45 fungicides induced an increase guaiacolperoxydase (GPX) and ascorbateperoxydase (APX) content in maiz root. These results agree With Avinash and Hoshmani [5]. Mittelstaedt *et al.* suggested that nuclei and mitochondria act as major targets of toxic action, probably by increasing the generation of free radicals Thioredoxin peroxidase, an enzyme that can neutralize hydrogen peroxide. Thioredoxin peroxidase-1 requires reducing equivalents from thioredoxin. Thioredoxin is in turn maintained in the reduced state of thioredoxin reductase activity and NADPH.

CONCLUSION

The present results concluded that these concentrations of Amistar and Dithane M-45 fungicides suitable for germination of seedlings, but concentration higher than these recommended can be unsuitable for germination of seedlings. The treatment of durum maize with the systemic fungicide Artea reveals that it could induce negative metabolic and biochemical changes which corroborate the toxic effects of fungicide on the plant

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