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Effects of Methyl Thiophenate (Systemic Fungicide) on Germination, Seedling Growth, Biomass and Phenolic Content of Resistant and Susceptible Varieties of *Triticum aestivum* L.

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Abstract: Effects of Methyl-thiophenate, systemic fungicide on germination, seedling growth and biomass of resistant and susceptible variety of *Triticum aestivum* were studied. Systemic fungicide's seed dressing increased the germination of both resistant and susceptible seeds. Application of systemic fungicide had greater effects on root and shoot growth of susceptible variety as compared to control. Root and shoot phenols were substantially elevated in both tested varieties following treatments with systemic fungicide particularly at higher concentrations (1500 and 2000ppm). Resistant variety was less affected in terms of fresh and dry weights as compared to susceptible, exhibiting some degree of tolerance to systemic fungicide.

Key words: Systemic fungicide, germination, growth, biomass, phenols, varieties

Introduction

All wheat cultivars grown commercially in most parts of the world are suspected to seed borne infection and losses are controlled, in part by chemical treatment of fungus at harvest or before planting. Topsin-M (Methyl-thiophenate) a systemic, curative and protectant fungicide is prepared by the reaction of sodium or potassium thiocyanate with chloroformic methyl ester and intermediate react with O-phenyl derivative to form methyl thiophenate (Handa, 1991).

The thiophenates are not themselves fungicides but are converted into active benzimidazole derivative thiabendazole (TBZ). Early in 1970s the benzimidazole fungicide thiabendazole was introduced for potato tuber and had proven to be very effective for the control of dry rot of seed and stored potatoes (Leach and Webb, 1981).

Both methyl and ethyl thiophenates are effective against diseases such as powdery mildew, apple scab, sheath blight of rice, leaf spot of cercospora, smut and rust of wheat (Thomson, 1985; Sobti, 1993). Despite their enormous application of thiabendazole and benzimidazole concern has been expressed about their phytotoxicity. There are several investigations to suggest that the use of systemic fungicides caused significant change in chlorophyll (Ahmed and Siddiqui, 1995), morphology (Reyes, 1975), germination and seedling growth (Siddiqui *et al.*, 1997), chromosomal number, structure (Coman *et al.*, 1990) and cell division (Pillonel, 1993).

Though most of the systemic fungicides are used as seed dressing yet several reports have shown phytotoxic effects produced by systemic fungicides on various plants. But most of the studies were restricted to examine the biochemical changes caused by fungicidal spray. Therefore, main objective of present investigation was to examine the effect of systemic fungicide's seed dressing on germination, seedling growth, biomass and phenolic contents of two varieties of *Triticum aestivum* (L.). Phenols were selected as stress indicator as it is known that toxic chemicals and various kinds of stresses lead to elevated total phenols in plants (Reid *et al.*, 1992).

Materials and Methods

Seeds of *Triticum aestivum* L. var. Kiran-95 (resistant) and Sarsabz (susceptible) were collected from Pakistan Agricultural Research Center (PARC) and soaked in a beaker containing 0, 1000, 1500 and 2000 ppm solution of systemic fungicide (Methyl thiophenate) for 45 min. Experiment was performed in 9cm diameter sterilized petri plates having Whatman No. 3 filter paper soaked in fungicide solution. Fifteen seeds of both varieties were placed in each petri plate separately. The solution of fungicide was based on formulation of active ingredient (thiabenzimidazole). Untreated filter paper served as control. Seeds were germinated under controlled conditions (29 ± 2°C during 13 hr light period from 6 a. m. to 7 p. m. of about 8000 lx and 24 ± 2°C during 11hr darkness). A seed was considered germinated when radicles had attained a length of not less than 1.5mm (Taylor, 1942). Small amounts of respective fungicide's solution were added, when it was obvious that petri dishes were beginning to dry out. After the completion of seed germination, root and shoot length of all germinated seedlings were measured, placed in an oven at 80°C for 24 hours and dry weights were recorded. A 50 % tolerance level (TL_{50}), the concentration at which seedling growth was reduced to 50 percent was computed using formula adduced by Davis *et al.* (1972) as follows:

$$TL_{50} = C_1 + [(C2 - C_1)(50 - P_1)] / (P_2 - P_1)$$

Where C_1 = highest concentration giving less than 50 % growth reduction, C_2 = lowest concentration giving more than 50 % growth reduction, P1 = percentage of growth at C_1 and P_2 = percentage growth at C_2.

The phenolic contents of dry root and shoot were measured using Folin Ciocalteu reagent (1:1) by the method of Swain and Hills (1959), after preparing the extract in 2N HCl. The data sets were subjected to factorial analysis using SPSS (a software package).

Results and Discussion

Effects on germination: Use of systemic fungicide methyl thiophenate showed significant (LSD = 5.732; $P < 0.001$) increase in germination of resistant variety over control (Fig. 1a). Methyl thiophenate did greater effect on susceptible variety of *T. aestivum* showing 100, 95 and 95 percent germination at 1000, 1500 and 2000ppm of fungicide as compared to 90, 100 and 100 percent germination recorded in resistant variety at 1000, 1500 and 2000ppm respectively.

Effects on seedling growth: Application of methyl thiophenate

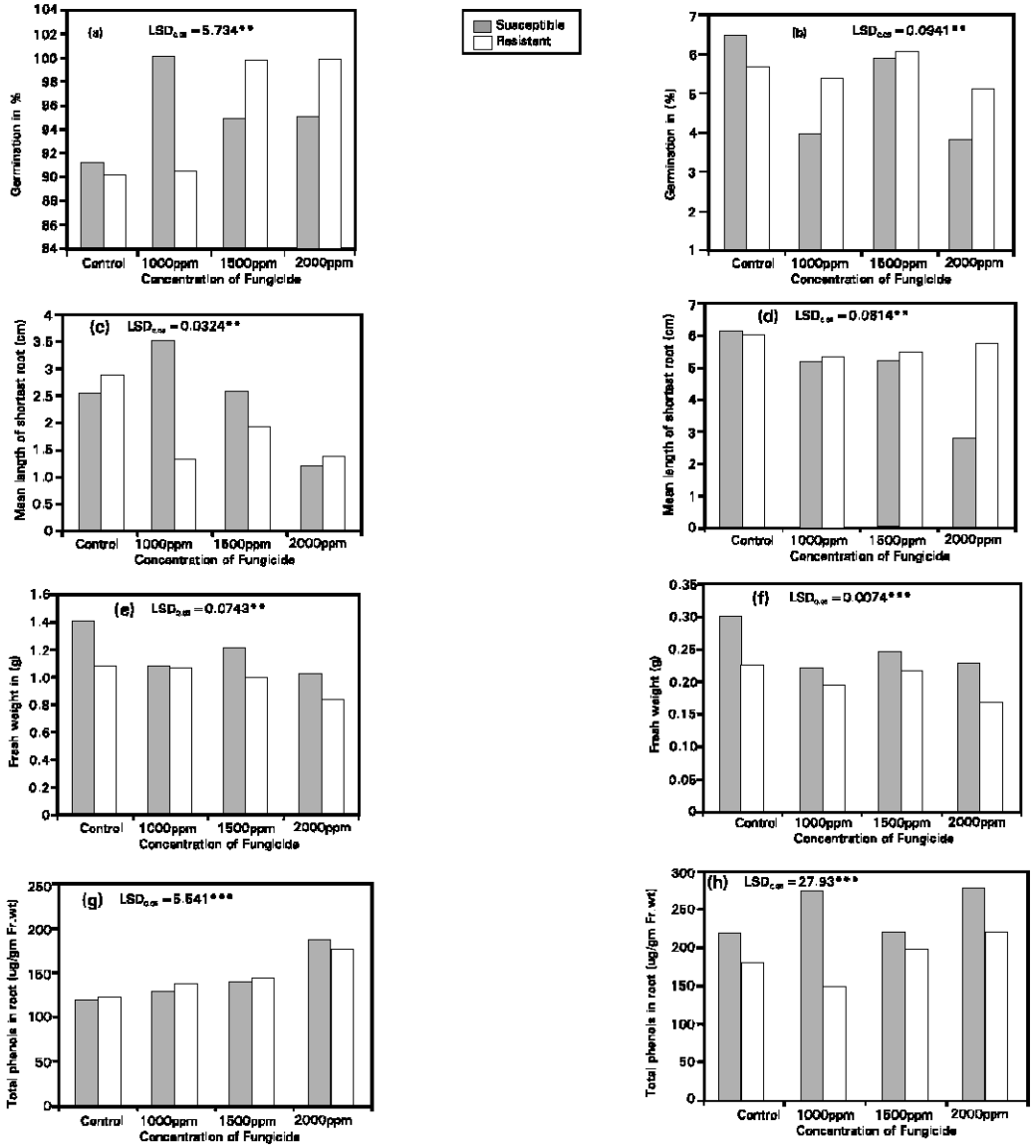

Fig. 1: Effect of Methyl-thiophenate (Topsin-M) on germination, seedling growth, biomass and phenolic content of resistant and susceptible variety of *Triticum aestivum* L. Symbol stand for (a) Germination (b) Shoot length (c & d) Root length (e & f) Fresh and Dry weights (g&h) Root and Shoot phenols. ** Significant at $P<0.01$ and *** Significant at $P<0.001$.

did some significant ($P<0.001$) effects on seedling growth (Fig. 1b, c & d). Root growth was more adversely effected than shoot growth. However, shoot and root growth reduced significantly by the use of fungicide. Root growth of susceptible plants was retarded more than the root and shoot growth of resistant plants. Maximum decrease (54.7%) was recorded in root growth of susceptible variety, when treated with methyl thiophenate at 2000ppm

Effects on biomass: Increasing concentration of fungicide results in the reduction of fresh weight of both varieties of *T. aestivum* (Fig. 1e & f). The dry weights of treated samples also followed similar pattern. Resistant variety seems to be affected more by application of fungicide than susceptible variety.

Phenolic content: Application of systemic fungicide showed significant ($P<0.001$) impact on total phenols of both varieties as compared to control (Fig. 1g & h). In both varieties, total root phenols followed a gradual pattern, whereas in case of shoot the increase was somewhat irregular. However, compared to control, the total phenols in shoot were high at 2000ppm of methyl thiophenate. Observation also showed that at 1000ppm application of fungicide the phenols in shoot markedly differ between two varieties. At the other concentration the difference was not as distinct.

TL_{50} values: For shoot growth of resistant variety values ranged from 1097.4 to > 2000ppm. While in case of root growth it was < 1877.22ppm. TL_{50} for root growth of susceptible variety was < 1045.43ppm and < 2000ppm for shoot growth. Germination percentage in both the varieties showed fluctuating patterns. An increase in germination values compared to control may be a specific response of *T. aestivum* to the treatment of fungicide. Although methyl thiophenate has been shown to cause an increase in chlorophyll, protein and phenolic contents of *C. annum* and *H. esculentus* (Ahmed and Siddiqui, 1995), however, such an increase in germination, as observed in present study has not been reported previously. The phenomenon also underscores the need for a comparative study of germination behaviour of different species in response to a particular fungicide.

Growth parameters viz. fresh and dry weights, length of shoot and (longest and shortest) roots showed a decrease as compared to the plants subjected to control treatment. Notable point is that in both the varieties enhanced fresh and dry weight and root and shoot length were observed at 1500 ppm application of methyl thiophenate then either at 1000ppm or 2000ppm applications. This may be suggestive of a minimum threshold limit of fungicide. Use of methyl thiophenate has also been found to cause an increase in fresh and dry weight of *Sesbania sesban* at concentration 0.25g/l (Siddiqui *et al.*, 1997). Phytotoxicity due to some abnormality in the metabolic pathway of plants may be the cause of reduced growth of treated plants.

In a study by Henry and Sisler (1979), a fungicide myconozole, although prevents the multiplication of sporidia of *Ustilago maydis*, but at higher concentration also checks the dry weight increase. The study goes on to suggest that a secondary toxicity mechanism non-operative at less concentration may become operative at higher concentration to cause a reduction in growth parameters like fresh and dry weight and root and shoot length.

Increase in total phenols at higher concentration in present study provides further insights to the reduction in growth parameters discussed above. Production of phenols in the plants subjected to the fungicidal spray is a response of the plants that not only helps them to cope with the resulting chemical stress but at the same time act as protective compound to check the growth of invaded pathogens. Reid *et al*. (1992) also reported an increase in phenolic contents like flavones under stress conditions. Friend (1977) suggested that these phenolic compounds also provide protection against pathogens.

The phytotoxin in the form of phenols have been found to have an adverse affect on nodulation, respiration, cell division, photosynthesis, disruption of cell membranes (Datta and Sinha- Roy, 1975; Macias *et al.*, 1992; Hafeez *et al.*, 1988). Similar effects of fungicide on chlorophyll, carbohydrate and mineral contents of several plants (Berger and Cwick, 1990; Pellisier *et al.*, 1971; Ahmed and Siddiqui, 1995) have previously been reported.

In present study too, concentration of methyl thiophenate as high as 2000ppm seems to have taken its toll upon the fresh and dry weight and root and shoot length and thus overall plant growth. Heisy (1990) has proposed that chemical stress in the plants exposed to fungicides facilitate the production of compound that are inhibitor of germination and seedling growth.

It is concluded that the plants can tolerate a cut off range of systemic fungicide concentration and show a normal or enhance growth due to the suppression of the pathogen growth, but once this cut off range is exceeded, the phytotoxin so formed to combat the pathogen attack, also begin to retard the plant growth parameters. It is therefore, necessary to use of these fungicides with the objective merely controlling the attack of pathogens rather than to eradicate it by indiscriminate application.

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