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# EFFECTS OF BENLATE SYSTEMIC FUNGICIDE ON SEED GERMINATION, SEEDLING GROWTH, BIOMASS AND PHENOLIC CONTENTS IN TWO CULTIVARS OF *ZEA MAYS* L.

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**Abstract**

Effects of benlate systemic fungicide on seed germination, seedling growth, biomass and phenolic contents of *Zea mays* L. cvs. EV1018 and Gohar were studied. Seed dressing with systemic fungicide enhanced the germination of seeds of both cultivars with greater effects on root and shoot growth of cvs. EV1018 as compared to control. Phenolic contents in root and shoot substantially increased in both the varieties following treatment with systemic fungicide particularly at higher concentrations of 1000mg $L^{-1}$ and 2000mg $L^{-1}$. Gohar was less affected in terms of fresh and dry weights as compared to EV1018, exhibiting some degree of tolerance to systemic fungicide. Usage of systemic fungicides beyond a cut off range or threshold concentration proved counter-productive for crop yield.

## Introduction

Seed dressing with systemic fungicide is a conventional method used for the control of seed borne infection. Though most of the systemic fungicides are used as seed dressing yet there are several reports showing phytotoxicity on various plants (Hack, 1994; Holderness, 1990; Kaspers *et al*., 1987). Most of the studies have been restricted to examine the biochemical changes caused by fungicidal spray (Reyes, 1975; Hack, 1994; Holderness, 1990; Siddiqui & Ahmed, 2002). The main objective of the present investigation was to examine the effect of benlate systemic fungicide as seed dressing on seed germination, seedling growth, biomass and phenolic contents of two varieties of *Zea mays* L. Phenols were selected as stress indicator as it is known that exposure to toxic chemicals and various stresses lead to elevated total phenols in plants (Reid *et al*., 1992).

## Materials and Methods

Seeds of *Zea mays* L. cvs. Gohar and EV1018 were collected from Pakistan Agriculture Research Centre (PARC) Karachi University campus Karachi and soaked in a beaker containing solution of benlate systemic fungicide for 60 min. Fifteen seeds of both the varieties were placed in Petri plates having Whatman No. 3 filter paper. Seeds soaked in distilled water served as control. Seeds were germinated in a Hotpack Refrigerated Incubator under controlled conditions (20 ± 2°C during 11 hrs light periods from 5 a.m. to 4 p.m. of about 25 µmole $m^{-2}$ $s^{-1}$ white florescence light and 15 ± 2°C during 13 h darkness). A seed was considered germinated when radicles had attained a length of not less than 1.5 mm (Taylor, 1942). Five ml of distilled water was added in the plates at two days interval, Seven days after seed germination, fresh weight, root and shoot length of all seedlings were measured. The seedlings were 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% was computed using the following formula adduced by Davis *et al*., (1972).

$$TL_{50} = C_1 + \{(C2 - C_1)(50 - P_1)\}/(P2 - P_1)$$

where $C_1$ = highest concentration giving less than 50% growth reduction,
$C_2$ = lowest concentration giving more than 50% growth reduction,
$P_1$ = % of growth at $C_1$ and
$P_2$ = % of growth at $C_2$.

The phenolic contents of dry root and shoot were measured using folin ciocalteu reagent (1:9) by the method of Swain & Hillis (1959), after preparing the extract in 2N HCl. The color was developed by treating the sample with 10% of Folin reagent. The treatments were replicated 4 times. The data sets were subjected to factorial analysis using SPSS.

## Results

**Effect on germination:** Use of benlate systemic fungicide showed significant increase in seed germination (Fig. 1a). Maize var. Gohar showed 100, 95 and 95% germination at 1000, 1500 and 2000 mg $L^{-1}$ while corresponding germination percentages for EV1018 variety were 90, 100 and 100%.

**Seedling growth:** Application of benlate considerably affected seedling growth (Fig. 1b, c & d). Root growth was more adversely affected than shoot growth. However, shoot and root growth in Gohar variety reduced significantly. Root growth of Gohar plants was retarded more than the shoot growth. Maximum decrease (54.7%) was recorded in root growth of Gohar variety, when treated with methyl thiophenate at 2000 mg $L^{-1}$.

**Biomass:** Higher concentrations of benlate fungicide showed reduction in fresh weight of both varieties of *Z. mays* (Fig. 1e & f). EV1018 seems to be affected more by the application than Gohar variety at high concentration compared to lesser concentration.

**Phenolic contents:** 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, total phenols in shoot were high at 2000 mg $L^{-1}$ of benlate. Application of fungicide @ 1000 mg $L^{-1}$ the phenols in shoot markedly differ between two varieties whereas at other concentrations the difference was not as distinct.

**$TL_{50}$ values:** Shoot growth values of EV1018 ranged from 1097.4 to > 2000 mg $L^{-1}$ while in case of root growth it was < 1877.22 mg $L^{-1}$. $TL_{50}$ for root growth of Gohar was <1045.43 mg $L^{-1}$ and <2000 mg $L^{-1}$ for shoot growth.

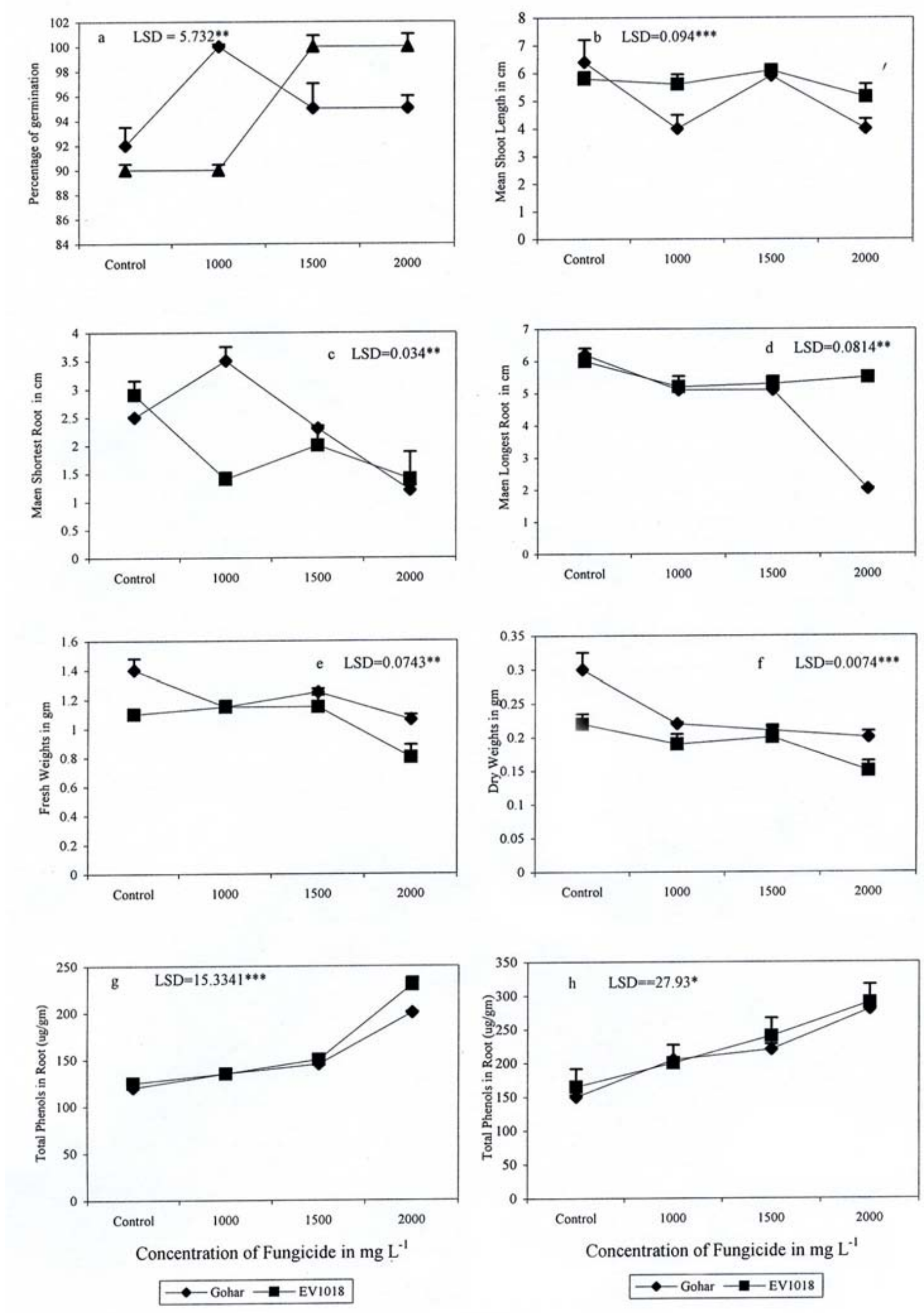


Fig. 1. Effects of benlate on germination, seedling growth, biomass and phenolic contents of *Zea mays* L. cvs. EV1018 and gohar. Symbol on graphs standfor (a) germination, (b) shoot length, (c & d) root length, (e & f) fresh and dry weights, (g & h) root and shoot phenols.
*=Significant at p<0.05, **=Significant at p<0.01 and ***=Significant at p<0.001.

**Discussion**

An increase in germination values compared to control may be specific response of *Z. mays* to the treatment of fungicide. Though benlate caused an increase in chlorophyll, protein and carbohydrate contents of *Capsicum annum* and *Hibiscus esculentus* (Ahmed & Siddiqui, 1995; Siddiqui *et al*., 1997), enhancement in germination as observed in the present study, has not been reported previously. The phenomenon also underscores the need for a comparative study of germination behavior of different species in response to a particular fungicide.

Increase in fresh and dry weight, root and shoot lengths of both cultivars were observed at 1000 mg $L^{-1}$ and 1500 mg $L^{-1}$ than at 2000 mg $L^{-1}$ applications. This may suggest as a minimum threshold limit of fungicide that can be tolerated by the plants. Use of benlate has also been found to cause an increase in fresh and dry weights of *Sesbania sesban* at 0.25g/l concentration (Siddiqui *et al.,* 1997). Siddiqui *et al.,* 1999 also reported the inhibition of seed germination and seedling growth in *Penesetum americanum* L. due to the application of organophosphate insecticides. Shive & Hugh (1976) showed that a fungicide triarimol not only prevented the gibberellin synthesis in *Phaseolus vulgaris* but also checked the dry weight increase over fresh weights at higher concentrations. The study suggests that a secondary toxicity mechanism non-operative at lesser concentration may become operative at higher concentration to cause a reduction in growth parameters like fresh and dry weight and root and shoot length. Windham & Windham (2004) have reported that systemic fungicides which are based on SBI (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. Use of high rates of systemic fungicides as seed treatments may also reduce the growth of small grains such as barley and wheat.

Increase in total phenols at higher concentrations of systemic fungicide provides further insight to the reduction in growth parameters discussed above. Production of phenols in the plants subjected to 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). It is presumed that increase in total phenols may act as prophylactic measure against pathogens before invasion. On the other hand phytotoxin in the form of phenols have been found to have an adverse affect on germination and growth parameters (Hafees *et al*., 1988; Macias *et al*., 1992; Berger & Cwick, 1990; Ahmed & Siddiqui, 1995; Siddiqui *et al.,* 1997). In the present study too, concentration of benlate as high as 2000 mg $L^{-1}$ seems to have taken its toll upon the fresh and dry weight and root and shoot length and thus overall plant growth. The study by Heisy (1990) has proposed that exposure of plants to fungicide create chemical stress facilitating the production of compounds that are potential inhibitor of germination and seedling growth. Einhelig *et al*., (1970) found that coumarin derivative; Scopoletin inhibits dry matter production, leaf area expansion and photosynthesis in tobacco, sunflower and redroot pigweed. Likewise, phenolics compounds such as Chlorogenic, Ferulic and *p*-coumaric acids reduced stomatal aperture, leaf water potential and stomatal diffusive conductance in sorghum, soya bean, tobacco and sunflower (Einhellig & Kuan, 1971; Einhelig *et al*., 1985). Einhellig (1995) proposed that a primary effect of phenolic acid is on the plasma membrane, and this perturbation contributes to a number of physiological effects causing growth reduction. Further studies are needed to understand the specific effects of individual phenolic compounds as the ambit of the present investigation was confined to total phenols only.

In the present study it was found that plants can tolerate a cut off range of systemic fungicide concentration that show a normal or enhance growth due to the suppression of pathogen, but once the cut off range so exceeded, the phytotoxin so formed to combat the pathogen attack, also begin to retard the plants growth parameters. It is therefore, necessary to use these fungicides with the objective of merely controlling or limit of the attack of pathogens.

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