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Effects of Atrazine and Phenolic Compounds on Germination and Seedling Growth of Some Crop Plants.

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Abstract: The phytotoxicity of atrazine and three phenolic compounds (i.e., benzoic, p-coumaric and caffeic acids) was investigated. The germination of six test species was reduced by atrazine in the order: pearl-millet > wheat > turnip > carrot > corn > mustard. Whereas, the phenolic compounds affected the germination of pearl-millet in the order: caffeic acid > benzoic acid > p-coumaric acid. Atrazine in conjunction with the three phenolic compounds exhibited synergistic effect on the process of germination and seedling growth.

Key word: Phytotoxicity, atrazine, synergistic effect, phenolic compounds.

Introduction

Triazine herbicides are widely used against broad leafed weeds in crops and tree seedling nurseries (Aidhous *et al*., 1968; Anderson, 1977; Ross and Lembi, 1985). They are photosynthetic inhibitors and are activated by light, causing chlorosis and desiccation of green tissues. Atrazine, a chloroaminotriazine is an apoplastically translocated photosynthetic inhibitor herbicide. Leaching of atrazine in most soils is limited. Einhellig (1989) and Raveton *et al*. (1997) studied the transformation and detoxification of atrazine in seedlings. Phenolics are one of the many secondary metabolites implicated in allelopathy and accumulate in soils causing inhibition of both seed germination and overall growth of many plant species (Inderjit, 1998). Many other workers have also reported the presence of phenolic compounds in soils (Blum, 1996; Kobayashi *et al*., 1996; Hrykun *et al*., 1996; Regnier and Macheix, 1996; Inderjit, 1996; Janovicek *et al*., 1997). The water soluble phenolic compounds are added to the soil as they are leached from plant parts of allelopathic species or are returned to the soil in the decomposition process of such plants (Guenzi and McCalla, 1966a,b; Datta and Sinha-Roy, 1975; Shaukat *et al*., 1983; Tongma *et al*., 1998).

Einhellig (1987) reported that a combination of atrazine with ferulic acid (FA) was much more inhibitory than FA alone. Hamm (1984) obtained reduction in growth with 10 ppb atrazine, but combined effect of atrazine and FA was much more severe. Similar findings resulted from combinations of trifluralin and salicylic acid (Einhellig and Leather, 1988). Herbicides alone had little impact on growth, but had cooperative inhibitory effects in conjunction with phenolic acids (Wegher, 1986). Shaukat *et al.* (1999) reported the synergistic effects of 2,4-D and phenolic compounds on germination and growth of *Pennisetum americanum* (L.) Schumann. Such synergistic effects may be helpful in weed management under field conditions.

The objectives of this study were: 1) to evaluate the activity of atrazine against six crop plants, 2) to test the toxicity of three phenolic compounds against pearl-millet, and 3) to examine the interactive effect of atrazine and the phenolic compounds.

Materials and Methods

Effect of atrazine on germination and seedling growth of test species: Five different concentrations of atrazine (80% WP) viz. 10, 20, 40, 80, and 160 ppm. were prepared. A few drops of tween-20 per litre were added. Toxicity of these concentrations was tested against pearl- millet (*Pennisetum americanum* L. Schumann), wheat (*Triticum aestivum* L.), turnip (*Brassica napobrassica* Mill.), carrot (*Daucus carota* L.), corn (*Zea mays* L.) and mustard (*Brassica compestris* L.).

Twenty seeds were surface sterilized with 0.3 percent calcium hypochlorite for 5 minutes and rinsed in distilled water. They were then placed on Whatman No. 1 filter paper in 9 cm diameter sterile Petri plates. 5 ml of each concentration was added to the plates. Control plates received 5 ml of distilled water. Each treatment and control was replicated thrice. Experiment was performed in a growth chamber maintained at 25 ± 1°C. Light intensity at the top of Petri plates was 2 KLux (14 h day length). Germination counts were made daily and small amounts of appropriate solutions were added when it was obvious that Petri plates were drying out. Root and shoot lengths of the seedlings were recorded after 72 h.

Effect of phenolic compounds (benzoic, p-coumaric and caffeic acids) on germination and seedling growth: Three different dilutions of each phenolic compound viz. 10, 20 and 40 ppm were prepared. Surface sterilized seeds of pearl-millet were used for this experiment. 5 ml of test solution were added to each Petri plate containing 20 seeds. Germination conditions were the same as outlined above. Germination counts were made daily and root and shoot lengths of the seedlings were measured after three days.

Combined effect of phenolic compounds and atrazine: The interaction of atrazine with benzoic, p-coumaric and caffeic acids was determined by using the same experimental setup as described above. The mixtures contained 80 ppm atrazine along with 10, 20 and 40 ppm of each phenolic compound.

Statistical analysis: Factorial analysis of variance was performed using the program FANOVA developed by us in FORTRAN 77 after arcsine transformation of the percentage germination data. As a follow up of FANOVA, Duncan's multiple range test was used (Sokal and Rohlf, 1995). Programs were implemented on Pentium II microcomputer.

Results

Atrazine significantly inhibited the germination and seedling growth of all the test species (p at the most 0.05). Germination was reduced in the order: pearl-millet > wheat > turnip > carrot > corn > mustard (Fig. 1a-f). Root length was reduced in the order: turnip > wheat > carrot > pearl-millet > corn > mustard, whereas shoot length was reduced in the order: turnip > wheat > carrot > pearl-millet > corn > mustard (Fig. 2a-f). The percentage germination and the seedling growth of pearl-millet were both significantly decreased by phenolic compounds (i.e., p-coumaric, benzoic and caffeic acids). Caffeic acid was found to be most inhibitory to germination and seedling growth (Fig. 3a-c, 4a-c), while benzoic acid was least inhibitory. When both atrazine and phenolic compounds were applied to examine the interaction of these compounds on germination and seedling growth, the inhibitory effect was more pronounced due to synergistic effect (Fig. 5, 6). This synergistic effect was more obvious in the case of atrazine and caffeic acid combination.

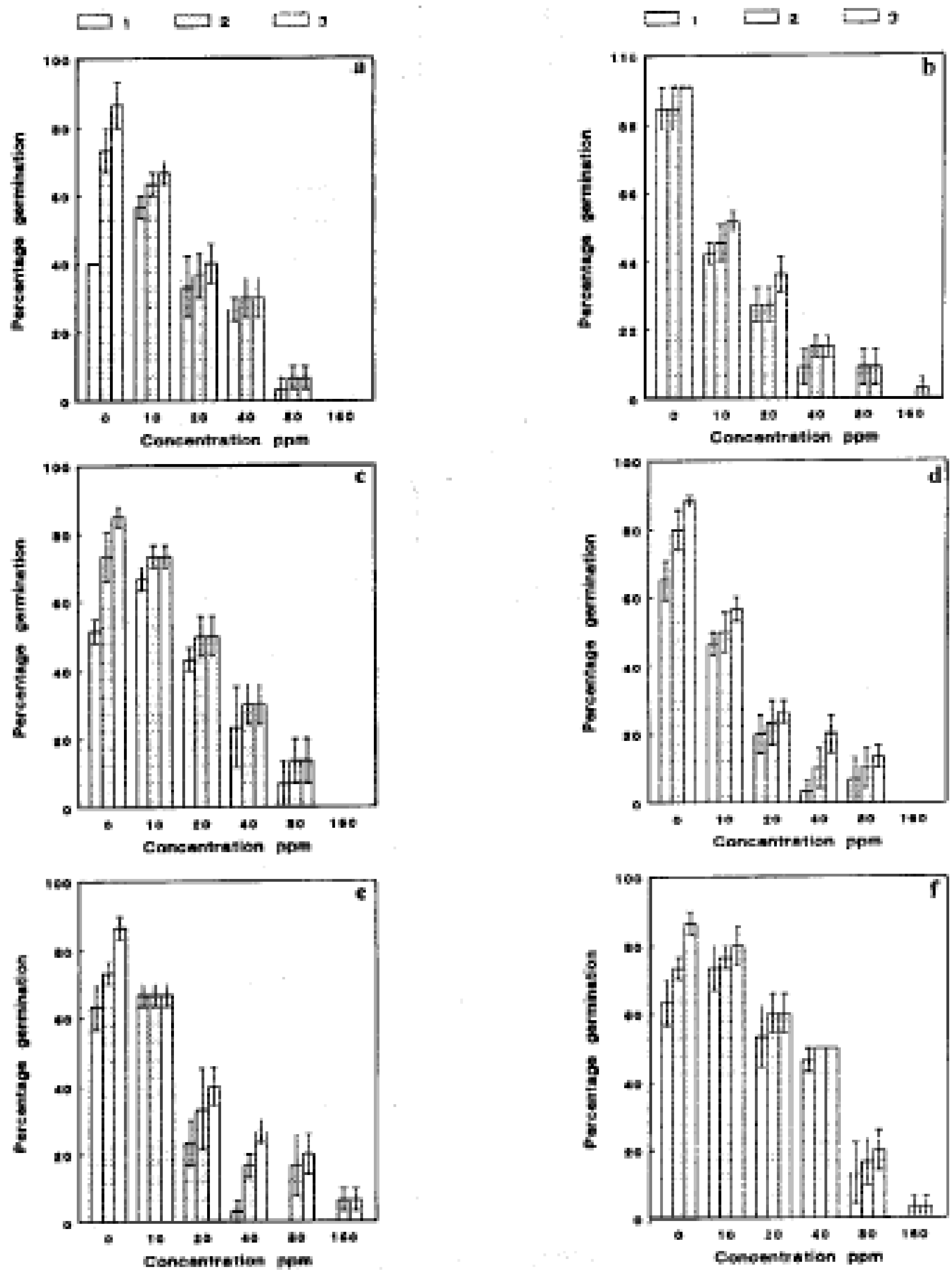


Fig. 1: Effect of atrazine on germination of test species a) pearl-millet, b) wheat, c) turnip, d) carrot, e) mustard, f) corn

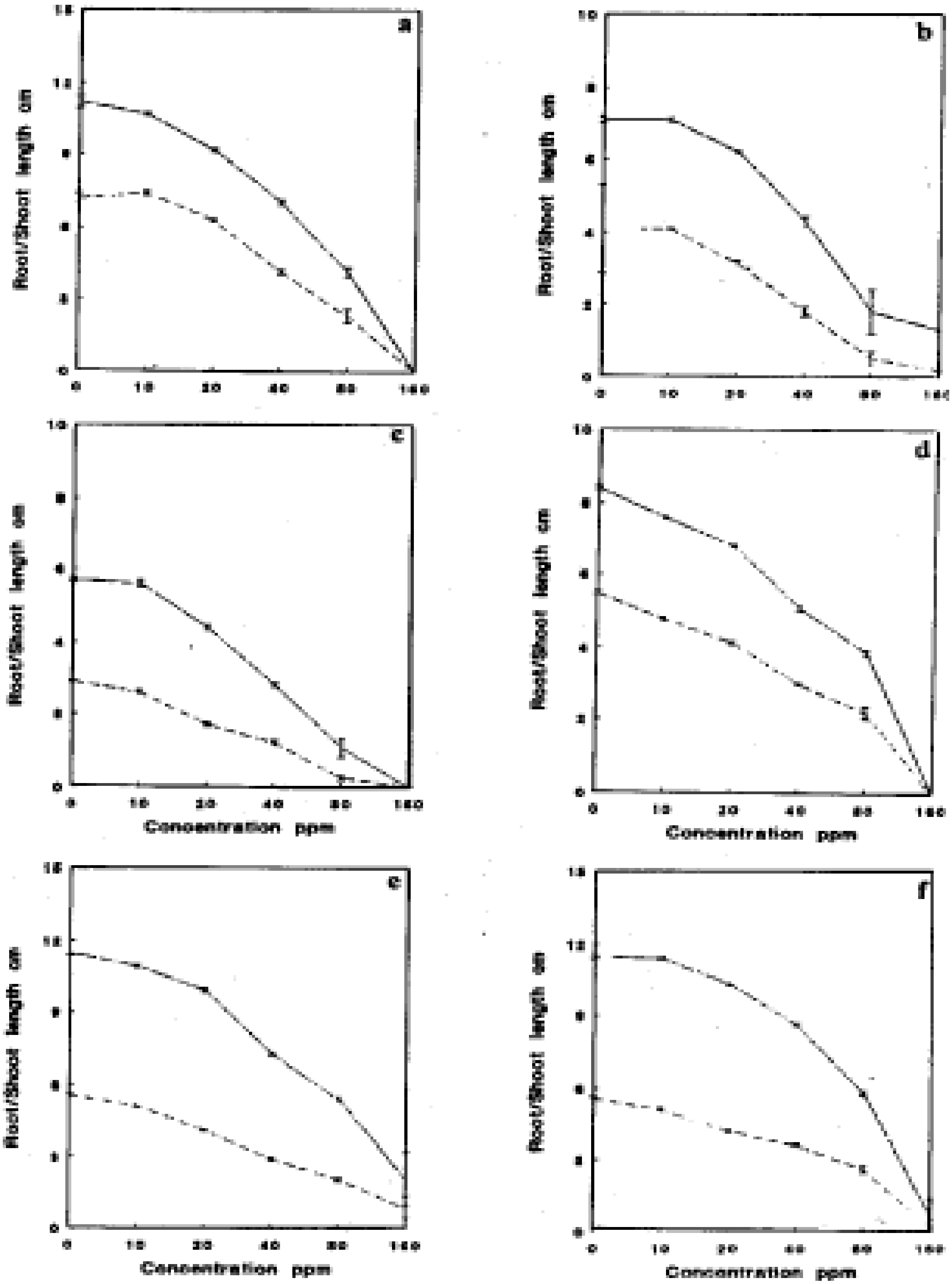


Fig. 2: Effect of atrazine on seedling growth of test species a) pearl-millet, b) wheat, c) turnip, d) carrot, e) mustard, f) corn

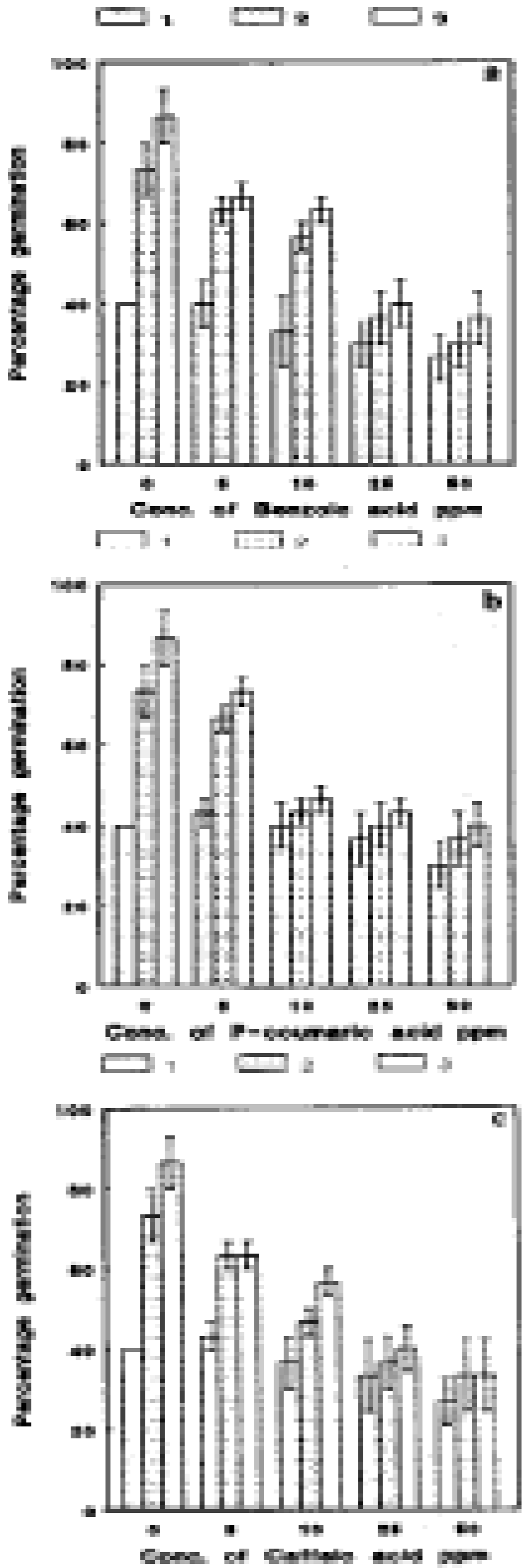


Fig. 3: Effect of phenolic compounds on germination of pearl-millet a) benzoic acid, b) p-coumaric acid, c) caffeic acid

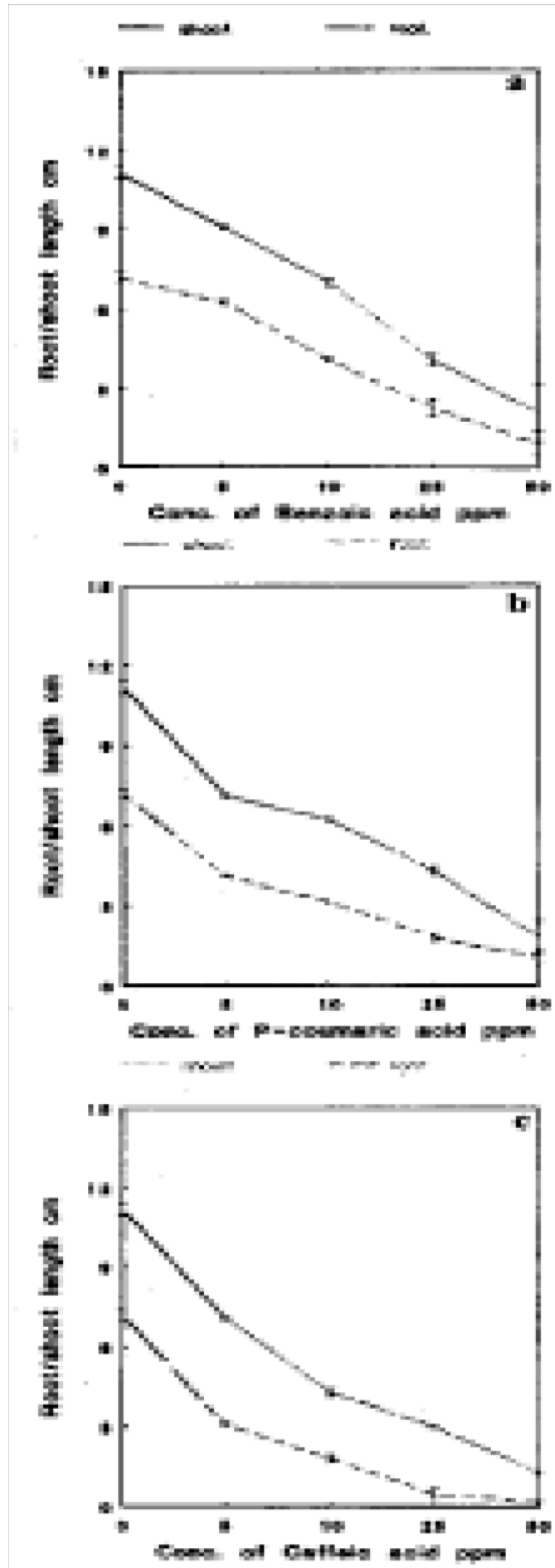
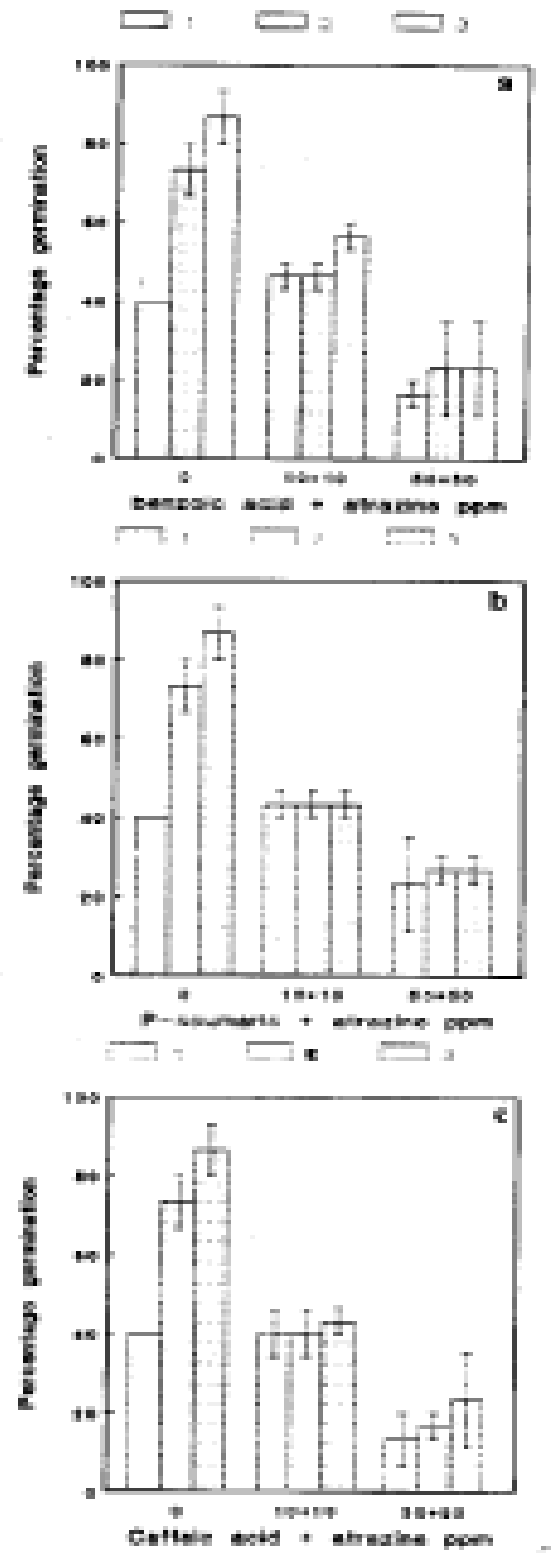


Fig. 4: Effect of phenolic compounds on seedling growth of pearl-millet a) benzoic acid, b) p-coumaric acid, c) caffeic acid



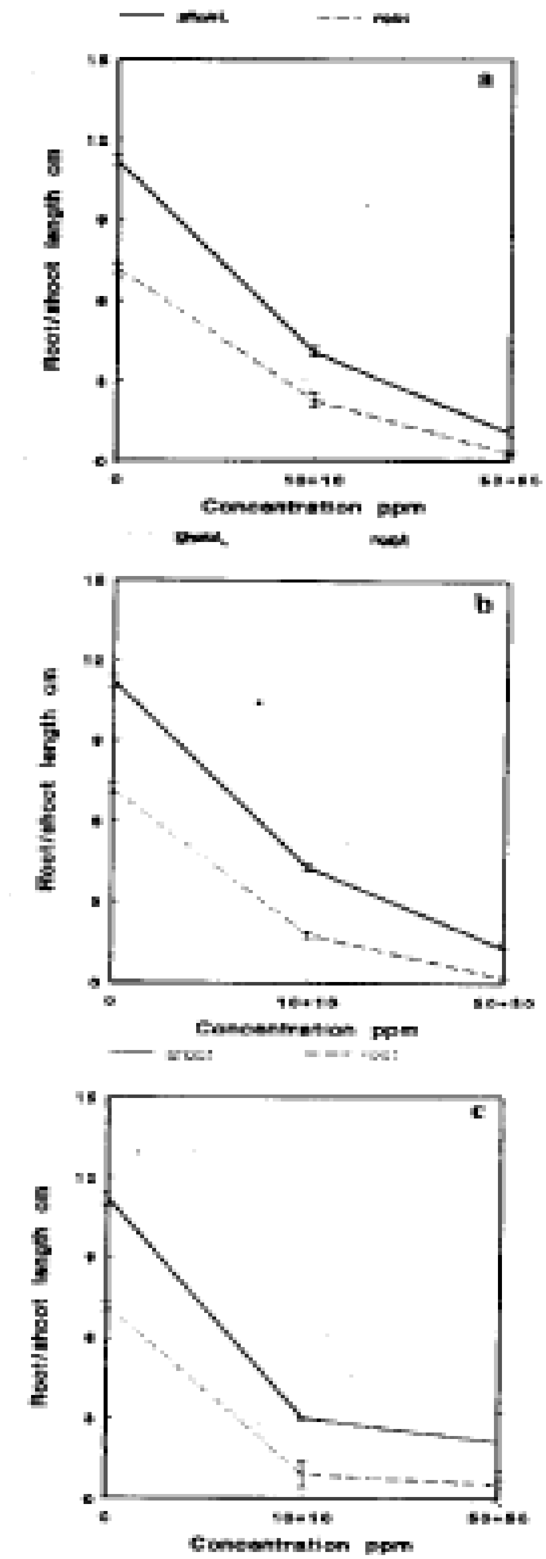


Fig. 5: Combined effect of atrazine and phenolic compounds on germination of pearl-millet a) atrazine + benzoic acid, b) atrazine + p-coumaric acid, c) atrazine + caffeic acid

Fig. 6: Combined effect of atrazine and phenolic compounds on seedling growth of pearl-millet a) atrazine + benzoic acid, b) atrazine + p-coumaric acid, c) atrazine + caffeic acid

Discussion

It is clear from the results that the herbicide atrazine is inhibitory to seed germination and seedling growth. Indiscriminate use of herbicides for weed control has resulted in very serious ecological and environmental problems such as increasing incidence of resistance in the weeds to important herbicides such as triazines (Forney *et al*., 1985). High concentrations of triazine herbicides such as atrazine and simazine can markedly reduce the germination percentage (Shaukat and Soni, 1974; Shaukat, 1976a; Bakke, 1936). The germination inhibition is presumably caused by the inhibition of amylase activity thereby suppressing the mobilization of reducing sugars and also due to the impairment of respiratory metabolism (Shaukat, 1976a).

All three tested phenolic compounds exhibited inhibition of germination and seedling growth. Tomaszewski (1960) found that p-hydroxy benzoic acid was present in 120 of the 122 species tested; caffeic acid was present in all but about a dozen species tested; p-coumaric acid occurred in all but three genera. Stowe *et al*. (1987) also observed inhibition of growth and germination of tested species by p-coumaric and p-hydroxy benzoic acid. Several workers have reported the inhibitory effects of phenolic compounds (Guenzi and McCalla, 1966a,b; Datta and Sinha-Roy, 1975; Rasmussen and Einhellig, 1977, 1979; Shaukat *et al*., 1983; Blum, 1996; Kobayashi *et al*., 1996; Regnier and Macheix, 1996; Inderjit, 1996; Janovicek *et al*., 1997). Most of the allelochemicals involved in plant-plant interaction are not as phytotoxic as commercial herbicides. Therefore, they are toxic only at very high concentrations (Duke and Lydon, 1987).

Combined effect of phenolic compounds and atrazine revealed synergistic action. Many other workers have reported that herbicide levels alone had lesser inhibitory effect on growth in comparison to the combined effect in conjunction with phenolic compounds (Shaukat, 1976b; Hamm, 1984; Wegher, 1986; Einhellig, 1987; Einhellig and Leather, 1988; Shaukat *et al*., 1999). This study emphasizes that the programmes of herbicide application in soil should also take into account the concentration and nature of phenolic compounds persisting in the soil because of possible synergistic action. Furthermore, during application in the field herbicide concentration may correspondingly be reduced which may substantially curtail the cost of chemical weeding.

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