DISCUSSION
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Seed germination and seedling shows promotory effects on these parameters in acid rain of higher pH (5.6) but a decrease was recorded at lower pH (2.5) except at pH 4.5. Acid rain of low pH may inhibit pH dependent enzymatic activities thus affecting seed germination and seedling growth. Seed germination, seedling emergence, establishment of seedling and plant growth are potentially among the most sensitive processes affected by acid precipitation (Abrahamsen et al., 1976, Lee and Weber, 1979). The present findings coincide are in agreement with those of Mayor et al. (1960), Boralker and Chaphekar(1981), Chand and Kumar (1987), Kumar et al., (1997). Such effects of acid rain with increasing age of plants were also noticed in certain legumes (Ashenden and Bell, 1989), Corn and sugarmaple (N-Soukpole et al. 1990), in Vicia faba (Nandita et al., 1992), in radish, spinach and bush bean (Hosono and Noushi, 1992) and in Pinus silvestrus (Malek, 1998). The reason behind this is that the acidic solution is absorbed into mesophyll of the leaf through stomata. Acute injury is caused by accumulation of sulphite and nitrate ions in the mesophyll tissue, which bring about metabolic changes such as destruction of chloroplasts and changes in the activities of enzymes. The cells are first inactivated with or without plasmolysis and are ultimately killed. When extensive areas get killed, the tissue collapses and dries up leaving a characteristic pattern of interval and marginal acute injury. If only a few cells in an area are injured, the area may become chlorotic owing to chronic injury (Thomas, 1961). Shiner (1977) reported that acid rain may also change the surface characteristic of foliage by erosion of epicuticular waxes. The erosion of epicuticular waxes may increase the wettability of leaf surface, thereby
enhancing the penetration of acidic solution. This leads to chronic injury manifested by brownish area on the leaf due to rupture of some cells or of chloroplasts within the cells.

In the present study it was observed that the mature leaves were more susceptible to damage by simulated acid rain than the younger leaves. Similar observations have been made on a variety of plant species (Ferenbaugh, 197; Evans et al., 1977, 1978; Evans and Curry, 1979; Paparozzi and Tukey 1983). This may be due to the fact that younger leaves being in synthetic phase (anabolic phase) can synthesize metabolites against the pollutants stress and hence resistant to acid rain. But since the mature leaves are in degrading phase (catabolic phase), degradation is accelerated by the acidic rain thus making them highly susceptibility to simulated acid rain.

The results of present study indicate that simulated acidic rain treatments cause considerable reduction in various growth parameters. At the end of the experiments, dry weight fractions of all the three test crops were found to be significantly reduced. These reductions were attributed to significant reductions in the number of leaves, root and shoot lengths.

Reduction were more significant at higher acidity (2.5) of rain. Similar result were obtained by several other investigators (Lee et al., 1981; Harcourt and Farrar, 1980; Johnston et al., 1982; Forsline et al., 1982; Forsline et al., 1983; Amthor, 1984; Norby and Luxmoore, 1983; Neufeld et al., 1985; Furumoto and Huttunen, 1991; Kost-Kurick and Manning, 1992; Singh et al., 1992; Allen et al., 1994; Khan et al., 2004).

In the plant cell, sulphites and sulphates of accumulated due to acid rain. Excess of $\text{SO}_4^{2-}$ leads to destruction of chlorophyll molecules (Ricks and
Williams, 1975). It may completely inhibit ribulose biphosphate carboxylase activity (Ziegler, 1974) and drop electron flow with in the chloroplast (Nieboer et al., 1976). These factors may be responsible for growth reductions in test crops. Decrease in phytomass accumulation eventually leads to decrease in net primary productivity (NPP). The reduction in photosynthetic rate as a result of acid rain is the major cause of reduction in the whole plant dry weight of Pinus armandi.

There are several reports which indicate that simulated acid rain do not cause any significant reductions in plant growth. On the other hand rain of low acidity showed slight increase in plant growth (Wood and Bormann, 1974). However, N’Soukpo-Kossi et al., (1990) reported that photochemical energy storage and the relative growth rate in sugar maple sapling were increased from pH 5.6 to 4.0-4.5 of acidic rain and decreased at pH 3.0 and then again increased at pH 2.5 of acid rain. Our observation, however, indicate that simulated acid rain at all pH values caused significant reductions in various growth parameters. Mohan and Kumar (1998) reported that damage to stomata and injury of membranes may be affected by acidic rain, which cause loss of nutrients through leakage. Thus water balance and hence growth is effected.

Acid rain of pH 4.5 was found to stimulated seed germination and plant growth. This response could be due to nutrient input effects primarily caused by nitrate present in simulated rain. Sulphate is not a limiting nutrient in most soils. Hence it may be possible that the soil got affected by sulphate fertilizations and added sulphate to the seedling. Similar findings were made earlier by Jacobson et al., (1986) and Kost Kurich and Manning (1992). According to Me Coll and Firestone (1991), acid rain has negligible deleterious effects on plant growth, on the treatment of intermediate acidity
contrary addition of N and S which act as fertilizers. Plocher et al., (1985) opined that moderate acid rain that contains nitrate and sulphate may actually increase the growth. Kumar (1997) and Verma and Prakash (1999), have made also similar observations.

Another important possible reason for growth reduction is that acidification of the cytoplasm may reduce auxin levels in leaves and cytokinin levels in roots, which in turn lower phytosynthesis (Wareing et al., 1968).

There was a significant reduction in fresh-and dry-weight fractions of shoot and root at pH 3.5 and 2.5. The decrease and increase in fresh and dry weight fractions were found to be associated with the shoot and root growth and numbers of roots. Acid rain interfere with the enzyme activity and as such low pH reduce growth and fresh-and dry-weight of plants (Boralker and Chaphekar, 1981). However, dry weight reduction in the present study was always higher in roots than in shoots. At lower acidity level sulphite ions in the soil have been reported to act as soil nutrients and as such stimulating plant growth and this eventually led to increase in dry weight fractions (Lee and Weber, 1979). Reduction in fresh and dry-matter accumulation were also associated with the reduction in the number and size of leaves, root and shoot lengths. These findings also find support from the observations of Harcourt and Farrar (1980), Troiano et al., (1982), Kumar (1997) and Verma and Prakash (1999).

The reduction in root biomass may also be due to slow translocation of metabolites in the roots due to reduction in photosynthetic activity by acid rain. Reduction in shoot and root dry weights were initially low but the final harvest after prolonged exposure significant decline in dry matter accumulation was
recorded. Okano et al., (1985), Ashmore (1988) and others have also made similar observations.

The net primary productivity and root weight ratio derived from plant biomass also recorded a reduction but there was an increasing trend in shoot weight ratio. The reduction in growth index, tolerance index and increasing in phytotoxicity percentage at low pH acid rain indicates more decrease in root lengths in comparison to higher pH treated plants. Such reduction got increased in progressive manner with the increasing concentrations of acidity. Similar observations have also been made by Ghouse and Khan (1984) and Narian and Singh (1984). Singh and Jain (1987) attributed these reductions to increased respiration and decreased photosynthesis. Ashenden (1979) made similar finding in Daetylis glomerata and Poa paratense after NO₂ exposure. Increase in shoot weight and decrease in root weight indicate that the rate of translocation of photosynthate to the roots is slowed down under the influence of acid rain which inhibit phloem loading system (Noyes, 1980; Teh and Swanson, 1982). Such a mode of phloem partitioning indicates that plants have greater priority to the growth of shoots than to roots under stress of pollution. Our results in this regards are similar to those of Mejstrick (1980), Shimazaki and Kiyashi (1980), Jensen (1981) and Gupta and Ghouse (1987).

A significant reduction in the number of stomata, number of epidermal cells, stomatal index, were observed in both the adaxial and abaxial surfaces of the leaf. The present observations agree with those of Satoh (1996), Kumar (1997) and Paoletti (1998) in this regard.

The number of stomata gradually decreased with the decrease in the level of pH (i.e. increasing acidity). Kumar also (1997) made similar observations. There were more stomata on the abaxial surface of the leaf than
on the adaxial surface. It was noticed that stomatal index a decreased while the number of epidermal cells increased due to acid rain treatments. Decrease in the number of stomata can be explained on the basis that the plants develop some sort of adaptive features to cope with the stressful effect of acid rain.

After entering the leaves, acid rain dissociate into H\(^+\), HSO\(_4\)^\(-\) and HNO\(_2\) ions and cause degradation of chlorophyll molecules by displacing Mg\(^{2+}\) ions by H\(^+\) ions from the tetrapyrrole ring of chlorophyll molecule resulting in the formation of photosynthetically inactive brown pigment, phaeophytin (Rao and Le Blanc, 1966, Malhotra, 1977; Shimazaki and Kiyashi, 1980). Accumulation of SO\(_4\)^\(-2\) and NO\(_3\)^\(-\) ions may lead to breakdown of chlorophyll and interaction between these acidic ions and chloroplast results in the inhibition of metabolic activity of the chloroplast. Hindawi et al., (1980) observed loss of chloroplast integrity in the injured leaves of *Phaseolus vulgaris* as a result of acid rain. Foliar chlorophylls also get affected to varying degrees depending upon the extent of acidity and duration of exposure to simulated acid rain. Loss in chlorophyll a, chlorophyll b and total chlorophyll was observed in all the three crops taken for the present study due to simulated acid rain. Reduction in chlorophyll a was always found to be more than chlorophyll b in treated plants. Higher susceptibility of chlorophyll a to acid rain has also been reported by some earlier workers (Khan and Khan, 1994; Verma 1999; Liu Jin Xiang et al., 2005). The reduction in chlorophyll a and chlorophyll b was dependent on the pH of acidic rain, duration of treatment and response of the cultivar. Goswami (2002) also recorded reduction in chlorophyll a and chlorophyll b in SO\(_4\) treated plant. Decrease in the chlorophyll content was accompanied by the reduction in the synthesis of photosynthates (N-Soukpoe et al., 1990). Sheridan and Rosentretor (1973)
also observed loss of chlorophyll, particularly chlorophyll a, after exposure to acid rain and suggested it was as a result of lower photosynthesis. Chlorophyll reacts with acidic ions in three distinct ways, bleaching, phaeophytinization and the process responsible for a blue shift in the pigment spectrum (Nieboer et al., 1976).

It has been suggested that sulphate ions (SO₄²⁻) react with iron (Fe) in the chloroplast, thus interfering photooxidative phosphorylation which causes death of cells. Chlorophyll breakdown is also done oxidation by free radicals Shimazaki et al., 1980; Beauregard, 1991). Wellburn et al., (1972) has reported that sulphate and nitrate ions are responsible for swelling of the thylakoids within the chloroplast of broad bean.

The phenomenon of photosynthesis is known to be crucial to the discussion of any of the effects of environmental pollution on plants. Reduction in photosynthesis in plants exposed to acid rain is explained that it is due to competition for NADH²⁺ between the processes of nitric and sulphate reduction and carbon assimilation in the chloroplast (Hill and Bennet, 1970; Srivastava et al., 1975) through ultrastructure change at or close to the thylakoids and possible destruction of chlorophyll and destruction of membrane integrity Wellburn et al., 1972). The acidic nature of solution (pollutant) changes the electron flow and photo phosphorylation (Treshow, 1984). Other effects of acid rain on photosynthesis are due to the formation of an iron nitric oxide free radical complex that could inhibit enzymatic activity in proteins containing sulphydryl groups of histidine residues (Woolum et al., 1968).

The leaf extract pH also recorded a decrease in plants treated with in simulated acid rain. Byterowicz et al., (1986) also reported decreased leaf
extract pH in acid rain treated plants. Rao et al. (1985) also recorded lower leaf extract pH in SO$_2$ treated plants of Vigna sinensis. Some studies have indicated that H$^+$ exchange is the primary mechanism for cation leaching from the leaf surface (Wood and Bonnmann, 1975; Hindawi et al., 1980; Scherbatskoy and Klein 1983; Evans et al., 1985). Bytherowicz et al. (1986) reported that H$^+$ concentration in plant tissue increases when buffering systems are weakened and the change in pH of leaf tissue exposed to acidic fog can be correlated to leaf injury. Leaching due to simulated acid rain enhance H$^+$ ion penetration into the foliage, resulting in decrease of pH of leaf extract.

The changes induced by acid rain in biochemical and physiological processes reduce crop production. At final harvest there was significant decrease in almost all yield attributes. The reduction in economic yield was due to decrease in number of fruit plant$^{-1}$, number of seeds fruit$^{-1}$ and weight of seeds. The extent of loss in economic yield was directly correlated with the pH of acid rain. Reduction in yield due to simulated acid rain has been reported earlier by several workers (Lee et al., 1981; Mersie and Toy, 1986; Temple et al., 1987, Porter et al., 1987; Takemoto et al., 1988; N-Soukpo et al., 1990).

The plant exposed to simulated acid rain flowered about 2-5 days earlier than control plants. This may be due to the fact that under stress conditions plant are in hurry to complete their life cycle.