Pak. J. Bot., 46(1): 245-250, 2014.

EFFECT OF SIMULATED ACID RAIN (SAR) ON SOME MORPHOCHEMICAL ASPECTS OF MASH (*VIGNA MUNGO* L.)

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Abstract

The studies were conducted to evaluate the effect of simulated acid rain (SAR) at early plant growth on some morphochemical characters of two varieties of Mash (*Vigna mungo* L.) namely Mash 97 and Var. 95009. Different pH values were made by using H_2SO_4, HNO_3, and combination of both. The data revealed that low pH (3.5) of either sulphuric acid or the combination of H_2SO_4 and HNO_3 affected more severely at all parameters including number of leaves, shoot: root ratio, water contents of shoot and Potassium ion concentration. Whereas for a few parameters like plant height and number of branches the simulated acid rain of solution of pH 4.5 and 3.5 by using HNO_3 proved a bit better for plant growth, the root length was increased in case of SAR of solution of pH 3.5 by using H_2SO_4+HNO_3. Foliar application of SAR of solution of pH greater than 4.5 showed some improvement in crop growth due to fertilizer effect of solution's components.

Introduction

Industrial revolution accompanied by air pollution has become a major source of acid rain and posing a threat to the healthy existence of natural and cultivated ecosystems (Tripathi & Gautam, 2007; Dwivedi & Tripathi, 2007). Pakistan has a geographic area of about 76.60 million hectares and about 80% of Pakistan's population is completely dependent on agriculture. As Pakistan lies in arid and semi-arid regions of the world, the most cheapest and immediate source of irrigation is rainfall (Anonymous, 2010 and Ghafoor *et al*., 1990). The pH of non-polluted rainfall mostly in forested regions ranges from 5.0 to 5.6 (due to presence of carbonic acid by dissolution of CO_2 into water molecules). The most prominent components of acid rain are Sulphuric acid and Nitric acid derived largely from the combustion of fossil fuels (Trolano *et al*., 1982 and Wagh *et al*., 2006). Fossil fuels account for about 80% of energy consumption in Asia (Balasubramanian *et al*., 2007). Due to its abundance and easy recoverability, coal is now the main fuel of choice to overcome the ever increasing energy crisis especially electricity shortfall in Pakistan following India and China. This increase in coal combustion will also boast up the existing increase of greenhouse gases and oxides of sulphur and nitrogen, driven by the rapid growth of Asian economics, inefficiency of energy use, the reliance on coal as major energy supply and the rapid increase in the number of vehicles (Bhattacharya *et al*., 2004).

Large quantities of sulphur oxides and nitrogen oxides are emitted into atmosphere from chimneys of industrial plant and other sources causing profound deterioration of urban air quality resulting from industrialization, urbanization, economic growth associated with an increase in energy demands (Kabir, *et al.*, 2012). Sulphur dioxide (SO_2) reacts with Oxygen in air (O_2) to form sulphur trioxide (SO_3), in-turn SO_3 reacts rapidly with atmospheric moisture (H_2O) to produce sulphuric acid (H_2SO_4) (Li & Gao, 2002). Similarly oxides of nitrogen react with moisture in the air to produce nitric acid (HNO_3), which fall as acid rain (Jonathan, 1989). Nitric and sulphuric acid in acidic rainfall (wet deposition) resulted in pH values of less than four for individual rainfall events as reported by Gumninghan (1999). A fog in Southern California in 1986 reached a pH of 1.7 approaching the acidity of some solution of HCl used as toilet bowl cleaner (Jonathan, 1989). Acid from acid rain damage the forests, agricultural crops, corrode metal and certain stones. Historical Taj Mahal and many statues are affected by abrasive and corroding effects of acid rain (Hamid, 1994).

Mash bean (*Vigna mungo* L. Hepper), being an important bean crop in the Indo-Pakistan sub-continent (Shaukat, *et al*., 2013) is used as experimental crop because it is a cheap source of protein having great value for food, feed, fodder and green manure in addition to soil fertility. Mash is very sensitive crop to high concentrations of acid like many other pulses. There are several mechanisms by which acid rain influences plant growth, as by direct foliar injury or indirect by affecting root's soil-water relations (Hussain *et al*., 2006 and Tariq *et al*., 2006). Mandre & Klyshejko (1995) has observed a slight positive effect on the biomass formation of *Phaseolus vulgaris* plant which may be due to fertilizing effect of nitrogen in simulated acid rain. But foliar injury accompanied by affected growth due to high acidity of simulated acid rain (less than 3.0) is being reported by Hosono & Nouchi (1994).

Although the problem is not much severe in our country but to face future hazards, the objective of present study was to observe the effect of different pH levels of simulated acid rain composed of different acid combinations on early growth and morphochemical characters of *Vigna mungo* L. cultivars.

Materials and Methods

A pot culture experiment was conducted in net house of the Botanical garden, University of Agriculture, Faisalabad, to study the effect of simulated acid rain (SAR) by using Sulphuric acid and Nitric acid and their combination (having different pH values) on morpho-chemical aspects of two varieties of Mash (*Vigna mungo* L. Hepper) Viz. namely mash-97 (V1) and var.95009 (V2) during early growth. Mash was selected for experimental studies due to its short life cycle duration (about three months) and is generally argued that it can grow in every kind of soil.

Experiment was performed in earthen pots filled with 9 kg of sun-dried sandy loam or "Bhal" soil (having about equal quantities of sand and soil) lined with polythene bags. Artificial acid rain was applied thirty days after germination and the solutions for simulation of acidified rain were made up according to Zvara *et al*., (1990) from sulphuric acid (H_2SO_4) and Nitric acid (HNO_3) added to distilled water to give different pH. The pH of each solution was maintained by using latest digital pH meter. Required pH solutions were prepared immediately before application.

Spraying of simulated acid rain (SAR) was performed like procedure by Evans *et al*., (1981) using domestic hand-spraying unit and allowed to fall on the foliage as well as the soil.
The meteorological parameters at the time of application of simulated acid rain (SAR) were as follows:

Temperature	=	23.5^{o}C
Relative Humidity	=	85%

There were ten acid levels including control
Acid Level-0 = Control (Natural rain)
Acid Level-1 = SAR of solution of pH 5.5 by using H_2SO_4
Acid Level-2 = SAR of solution of pH 4.5 by using H_2SO_4
Acid Level-3 = SAR of solution of pH 3.5 by using H_2SO_4
Acid Level-4 = SAR of solution of pH 5.5 by using HNO_3
Acid Level-5 = SAR of solution of pH 4.5 by using HNO_3
Acid Level-6 = SAR of solution of pH 3.5 by using HNO_3
Acid Level-7= SAR of solution of pH 5.5 by H_2SO_4 + HNO_3
Acid Level-8= SAR of solution of pH 4.5 by H_2SO_4 + HNO_3
Acid Level-9=SAR of solution of pH 3.5 by H_2SO_4+ HNO_3

Single harvest was taken fifteen days after application of simulated acid rain to observe the morpho-chemical aspects under various levels of acidic rainfall. Plants for each treatment were uprooted in both varieties by removing soil with the help of gentle stream of tap water, causing least possible damage to the root system of plants. They were kept in polythene bags and brought to the laboratory for the study of parameters. Experiment was laid out in Completely Randomized Design (CRD) with four replicates. The data regarding morpho-chemical aspects of mash were collected and analyzed using Ducan's Multiple Range (DMR) Test following Steel & Torrie (1980).

Results and Discussion

The effect of simulated acid rain (SAR) was observed on different morphological and chemical parameters of mash crop *Vigna mungo* L. These parameters of research crop were measured and then the mean values of replicates were recorded. In general minute visual symptoms of foliar damage because of treatments of low pH i.e., 3.5 was evident in both varieties of mash as poor chlorosis and wilting of some old basal leaves. Reduction in plant height is reported by Balasubramanian *et al*., (2007). The data about plant height (Fig. 1a & b) revealed highly significant differences among different acid levels of simulated acid rain. Maximum plant height 26.19 cm was measured in plants of Acid level-6 (simulated acid rain of solution of pH 3.5 by HNO_3) and the minimum plant height 23.39 cm in case of Acid level-9 (simulated acid rain of solution of pH 3.5 by using combination of H_2SO_4 and HNO_3). Varieties into acid level interaction also differed significantly giving maximum value 28.78 cm in AL-6 of var.95009 while minimum value 20.0 cm was noted in AL-9. Interaction revealed that V2 (var.95009) behaved in better way under different acid level treatments of simulated acid rain as compared to V1 (Mash 97). Similar results were also inferred by Porter *et al*., (1989).

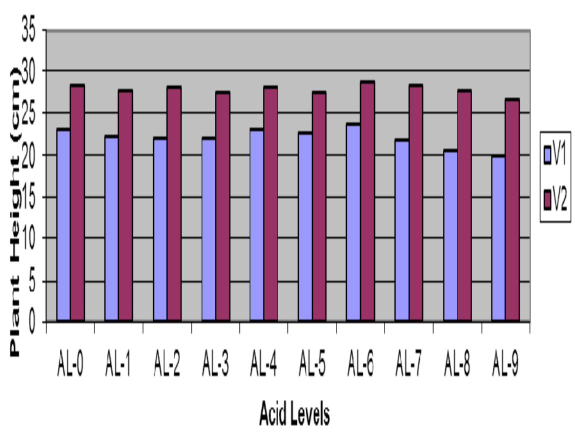

Fig. 1(a). Effect of SAR on plant height (cm).

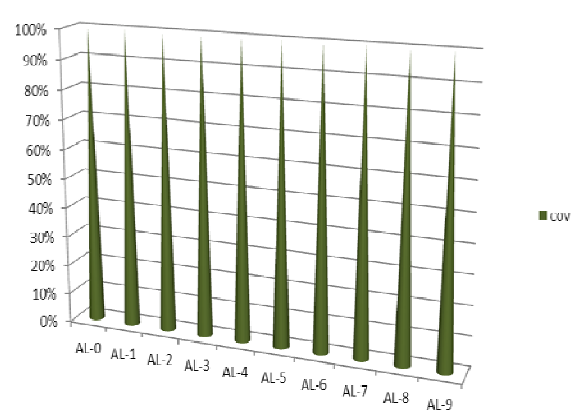

Fig. 1(b). Coefficient of variance (COV) for the effect of SAR on plant height (cm).

The data about root length (Fig. 2 a & b) also showed highly significant differences under different levels of SAR. The maximum value 18.47 cm was observed in AL-9 (SAR of pH 3.5 by using combination of H_2SO_4 + HNO_3), this increase in root length reveals the effect of acidity in the rooting medium and resultantly increase in length of root cells as well as indicates the resistance/avoidance as adaptive mechanism of this plant to cope with the high acidic levels by growing roots deeper in the soil where acidity level remains moderately low and stable, while minimum value 14.78 cm was shown by AL-3 (SAR of pH 3.5 by using H_2SO_4). Varieties into acid level interaction showed significant differences as maximum value 20.78 cm was observed in AL-9 of var.95009, while minimum value 11.25 cm noted in AL-3. Overall it is observed that acids in the combined form are more influential as compared to individual application of sulfuric and nitric acids in the form of simulated acid rain. The results are in agreement with Harcourt & Farrar, 1980 and Sant Anna-Santos *et al*., 2006).

The data about number of leaves (Fig. 3 a & b) also revealed highly significant differences in case of acid levels and varieties. As comparison of acid levels mean revealed that maximum number of leaves 8.96 was observed in AL-0 or control plants and minimum value 6.72 in AL-9. While varieties into acid levels interaction showed non-significant differences. Maximum number 9.53 was observed in AL-0 or control of V2 (var.95009) and minimum value 6.23 in AL-9 (SAR of pH 3.5 by using H_2SO_4 and HNO_3) of Mash 97 (V1). This confirms the stressful effects of acid rain on vegetative or foliar growth and biomass of plants. The results are similar to findings of Mandre & Klyshejko (1995), who reported that simulated acid rain, affects number of leaves by causing foliar damage.

Statistical analysis of data regarding number of branches (Fig. 4a & b) revealed highly significant differences between varieties, acid levels and varieties and acid level interaction. Comparison of acid level exhibited maximum value 2.30 in SAR of pH 5.5 by using HNO_3, while minimum value 1.35 was observed in SAR of pH 3.5 by using H_2SO_4, as 40.2% decrease was observed. This increase in number of branches depicts the slight fertilizer effect of nitrogen in case of nitric acid (HNO_3 application (in milder acidic pH). Varieties also showed highly significant differences as higher value 2.18 was observed in V2 (var.95009). Interaction of varieties into acid levels revealed that higher value 2.85 was observed in AL-4 (simulated acid rain of pH5.5 by using HNO3) of V2 (var. 95009), and the lowest value (1.28) in AL-9 (simulated acid rain of solution of pH 3.5 by using H2SO4 + HNO3) of Mash 97 (V1) refers to the harmful effects of acidic pH in the form of simulated acid rain on overall vegetative growth of plant.

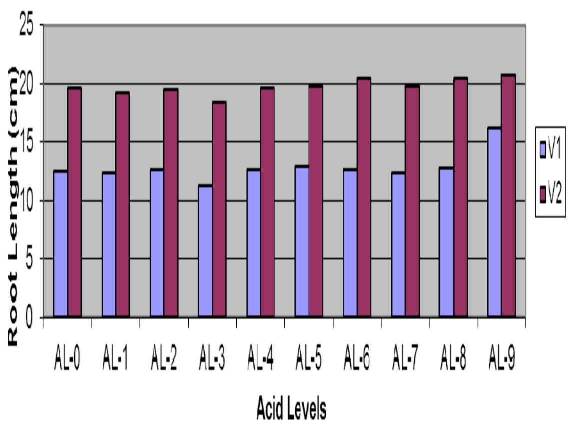

Fig. 2(a). Effect of SAR on root length (cm).

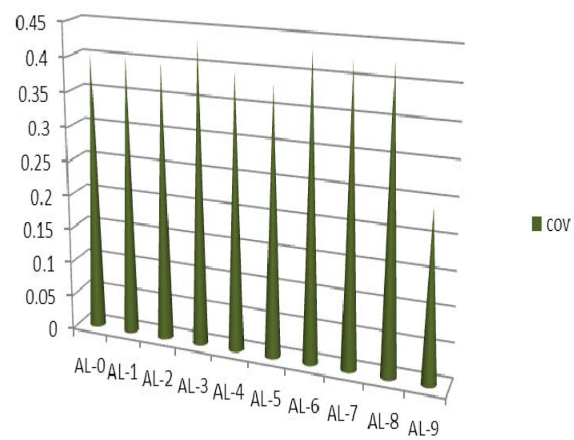

Fig. 2(b). Coefficient of variance (COV) for the effect of SAR on root length (cm).

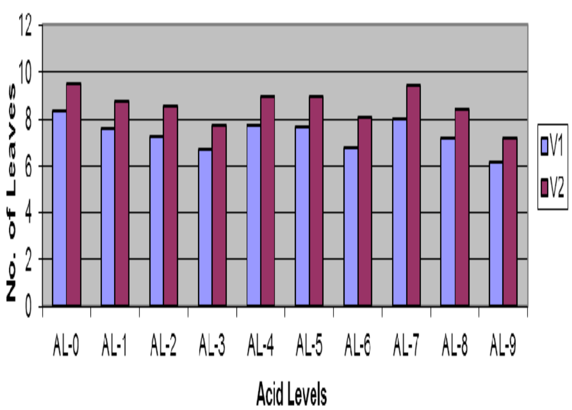

Fig. 3(a). Effect of SAR on number of leaves.

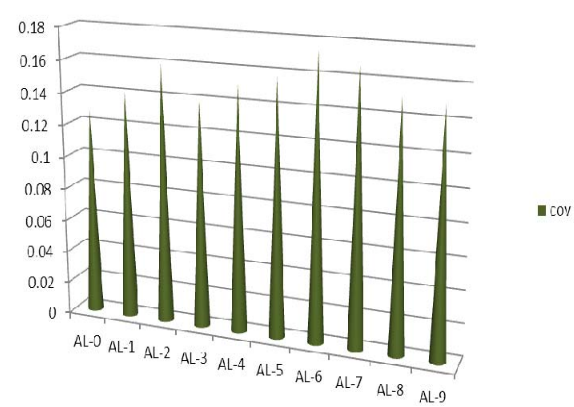

Fig. 3(b). Coefficient of variance (COV) for the effect of SAR on number of leaves.

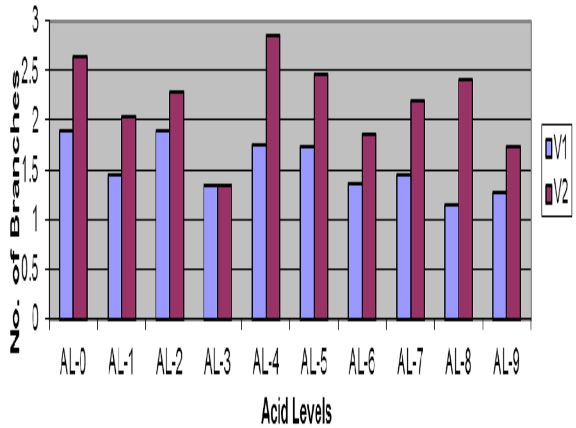

Fig. 4(a). Effect of SAR on No. of branches

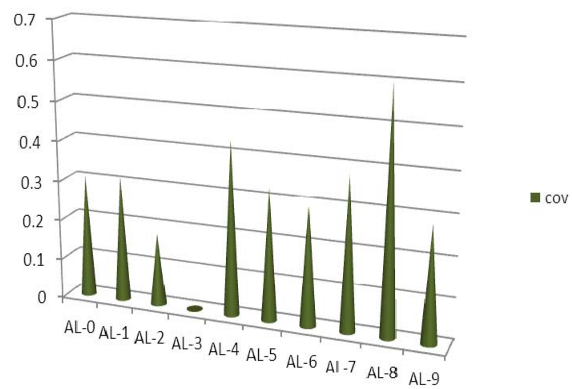

Fig. 4(b). Coefficient of variance (COV) for the effect of SAR on no. of branches.

Statistical analysis of data regarding the shoot: root ratio (Figs. 5 a & b) as affected by different acidic levels of SAR revealed that the variety Mash 97 (V1) showed maximum value for shoot: root ratio (1.74). Comparison of acid levels also showed highly significant differences. Max. value (1.72) was noted in AL-3 while minimum value for shoot: root ratio (1.27) was noted in AL-9. Varieties and acid levels interaction was also highly significantly different. The highest value (1.94) was given by V1 (Mash 97) under AL-3 and the lowest value (1.26) for shoot: root ratio was observed in AL-9 of V2 (var.95009). These results are in accordance with the findings of Porter *et al*., (1989) and Suneela & Thakre (2001).

The comparison of data regarding the effect of different acid levels in SAR on shoot water contents (Fig. 6 a & b) of mash varieties revealed that maximum value 74.83% was observed in AL-0 (Control) while minimum value (70.58%) in AL-5. Varieties showed non-significant differences which means both varieties behaved similarly under different acid levels of simulated acid rain. Varieties into acid levels interaction showed highly significant differences as maximum value 75.16% was observed in AL-0 of V1 while minimum value 68.16% was calculated in AL-3 of V1. As the acids in foliar application of simulated acid rain as sulfuric acid and nitric acid separately or in combination causes loosening of cell membranes and disrupts the balanced water contents of plants cells by lowering the overall plant water contents. These findings are in accordance with Singh & Agrawal, (2004).

Statistical analysis of data pertaining to Potassium ion concentration of shoot (Fig. 7 a & b) as affected by SAR revealed that varieties and acid levels showed highly significant differences while their interaction showed non-significant differences. Comparison of acid levels indicated that maximum value 9.43mg/g occurred in AL-6 while minimum Potassium ion concentration of shoot (7.07mg/g) was recorded in AL-9. On comparing varieties, it was noted that V2 gave a higher value for Potassium ion of shoot than V1. Interaction of varieties and acid levels showed non-significant differences. Maximum value 9.69mg/g was observed in AL-6 of V2 while minimum value 6.88mg/g in AL-9. These findings reveals the effects of acidity in the form of acid rain on the transport and ionic metabolism of important ions as higher acidic environment causes imbalance of metabolites including potassium ions in the cell cytosol as well as plant sap. These results are similar to the findings of Mandre & Klyshejko (1995), who reported decrease in Potassium ion concentration of shoot at low pH of solution sprayed as simulated acid rain.

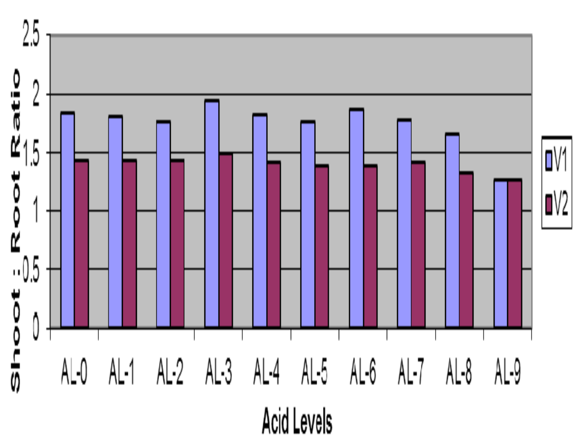

Fig. 5(a). Effect of SAR on shoot: root ratio.

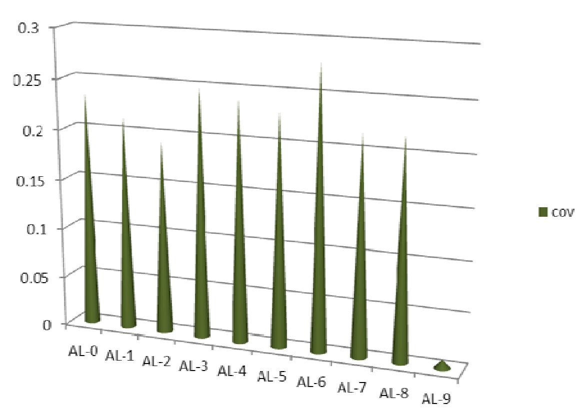

Fig. 5(b). Coefficient of variance (COV) for the effect of SAR on shoot: root ratio.

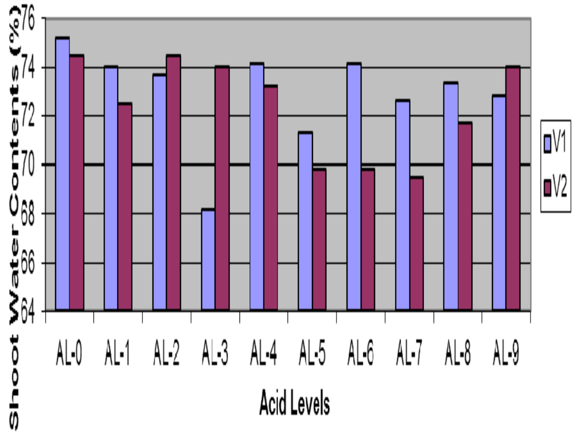

Fig. 6(a). Effect of SAR on shoot water contents (%).

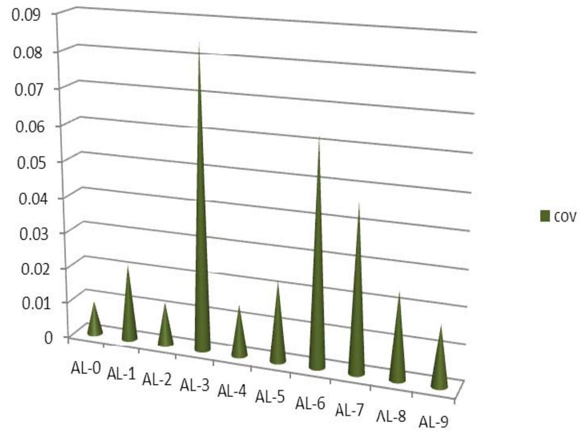

Fig. 6(b). Coefficient of variance (COV) for the effect of SAR on Shoot Water Contents (%)

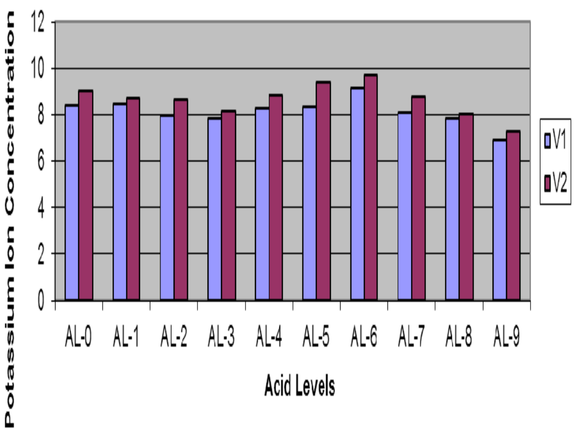

Fig. 7(a). Effect of SAR on potassium ion concentration.

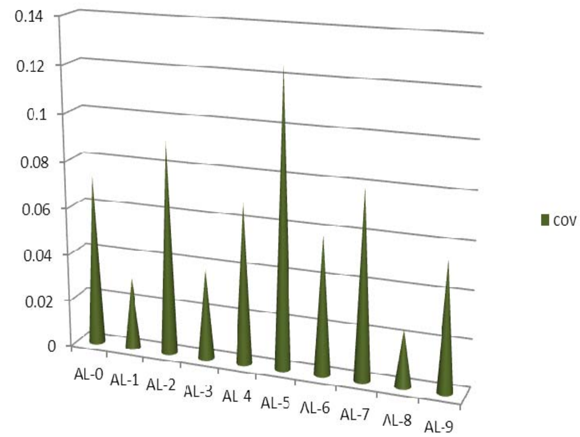

Fig. 7(b). Coefficient of variance (COV) for the effect of SAR on potassium ion concentration.

Conclusion

Statistical analysis of the data revealed that low pH (3.5) of either sulphuric acid or the combination of H_2SO_4 and HNO_3 affected more severely to all parameters including number of leaves, shoot: root ratio, water contents of shoot and Potassium ion concentration. Whereas for a few parameters like plant height and number of branches the simulated acid rain of solution of pH 4.5 and 3.5 by using HNO_3 proved a bit better for plant growth, the root length was increased in case of SAR of solution of pH 3.5 by using H_2SO_4+HNO_3 probably as physiological strategy to face acid stress plant roots grow lengthwise in deeper soil horizon to avoid acidic soil medium. Foliar application of SAR of solution of pH greater than 4.5 showed some improvement in crop growth due to fertilizer effect of solution's components especially in the form of nitrogen from HNO_3.

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(Received for publication 18 April 2012)