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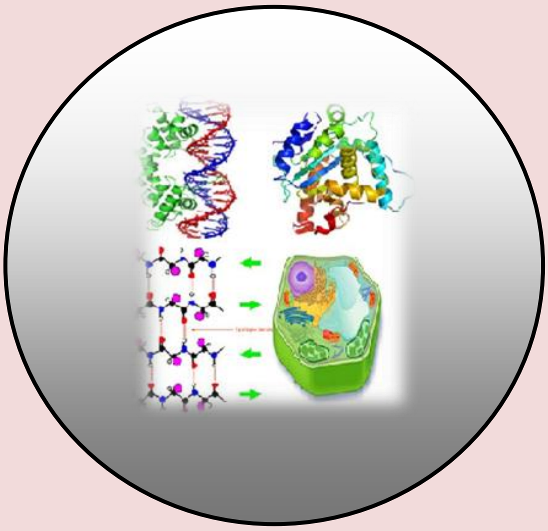

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RESEARCH PAPER

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# Effect of Simulated Acid Rain and Root-knot Nematode on Plant Growth, Yield and some Biochemical Substances in Pumpkin Crop

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**ABSTRACT**

***A pot experiment was conducted in green house to evaluate the effect of simulated acid rain and root-knot nematode (Meloidogyne incognita) on pumpkin. Simulated acid rain was prepared with concentrated sulphuric acid ($H_2SO_4$) and nitric acid ($HNO_3$) in a ratio 3:1diluted with double distilled water, to get the desired pH using a Deluxe pH meter. The 15 days old plants inoculated with nematode were exposed to simulated acid rain (pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0). The exposure treatments were given twice in a week for two months. The effect of simulated acid rain showed that higher pH levels (3.0, 3.5, 4.0, 4.5 and 5.0) induced morphological changes as chlorosis, early leaf senescence, necrosis, leaf folding and death. Plant height (root and shoot), plant weight (fresh and dry weight of root and shoot), yield (flower and fruit), leaf related water content, chlorophyll, protein and carbohydrate, NPK and leaf epidermal character of pumpkin were decreased gradually as the level of pH was increased as compare to control. Highest reductions in all above parameters were observed in 3.0 pH level. The harmful effect was acidity dependent.***
***Keywords: Chlorophyll, Chlorosis, Growth, Yield, Nematode and Pumpkin.***

## INTRODUCTION

Acid rain (AR) is one of the serious issues of environment. It is derived from the burning of fossil fuels and traffic emissions in the form of oxides of nitrogen and Sulfer (Abbasi, et al., 2013; Singh and Agrawal, 2008; Menz and Seip, 2004). AR shows negative impacts on human being, plants, animals and also on non-living things. In comparison to woody plants, most of the herbaceous plants showed high sensitivity and direct injury (Heck et al., 1986). By the deposition of AR, plants faces lot of problems like the various changes in the mesophyll, chloroplasts and mitochondria of the leaf (Odiyi and Bamidele, 2014; Wyrwicka and Skłodowska, 2006). Besides these it shows the harmful effects including chlorosis, necrosis, early senescence, stunting, loss of chlorophyll content, reduces the rate respiration and photosynthesis (Evans et al., 1997; Wyrwicka and Skłodowska, 2014; Zhang et al., 2015). Moreover, by decreasing the rate of photosynthesis and loss of chlorophyll content which impede the plant growth due to AR (Liu et al., 2015). The harmful effects of acid rain have been reported on several crops like wheat, tomato, soya bean, lentil and coriander, field corn, green pepper, tomato etc (Kausar et al., 2006; Kazim, 2007; Singh, 1989; Banwart et al., 1988; Shripal et al., 2000; Dursun et al., 2002). Root-knot nematodes (*Meloidogyne* spp.) are microscopic but very destructive polyphagous endoparasites that show the parasitism of diverse cultivated plants globally and it is a serious threat to food security worldwide. More than 110 species have been described and a large number of crops affected (Sasser and Carter, 1982; Khan and Khan, 1990; Khan and Siddiqui, 2005; Trudgill and Blok, 2001). The global yield loss due to this destructive plant parasitic nematode approximated as 25-50% over infected cultivated area of about 100 billion dollars annually and tomato shows about 24-38% (Trudgill and Blok, 2001). In host roots they complete their life cycle within 4-6 weeks and depend on environmental factors such as availability of a suitable host, temperature and moisture by secreting the effector molecules consist of enzymes, peptides, metabolites and biomolecules that maintaining the feeding cells for feeding (Abad and Williamson, 2010; Mitchum et al., 2013, Shukla et al., 2016). The most common species (*M. incognita*) estimated to be able to infect almost all the cultivated plant species. Pumpkin (*Cucurbita moschata* Duch. ex. Poir) is one of the important cucurbitaceous vegetable. It is cultivated throughout the tropical regions of India and almost all the countries of Africa, Asia and South America. In India, pumpkin is cultivated in an area of 11,060 hectares with the total production of 2.77 lakh tonnes which have productivity of 25.10 tonnes per hectare during 2014. The flesh and peel of the fruit represent rich sources of pectin-type dietary fiber and antioxidants (Caili et al., 2006). It is used as in immunological activity and other pharmacological activities such as lipid-lowering, hepatoprotective (Makni et al., 2008), anti-carcinogenic, anti-microbial (Caili et al., 2007; Park et al., 2010) and anti-diabetic properties (Adams et al., 2011; Caili et al., 2006; Xia and Wang 2006; Yadav et al., 2010). It is also used as anti-oxidant, reducing the blood pressure, reducing blood lipids, lowering cholesterol levels (Yong et al., 2006), especially for the treatment of diabetes (Zhang et al., 2002). This is crop considered highly susceptible to root-knot nematode. Therefore in the present study it was planned to observe the effect of acid rain and *M.incognita* together on pumpkin crop.

## MATERIALS AND METHODS

The experiment was carried out in a greenhouse at Aligarh Muslim University, Aligarh, India. The experiment was done in pots with diameter at the top was measured as 22 cm, bottom diameter 12.5 cm and height of pot 10 inches. The soil used in the experiment was collected from the unpolluted agricultural field up to 20 cm depth after scrapping the surface of litters present. The collected soil was brought to the laboratory in gunny bags and mix with farmyard manure, sand and soil at the ratio of (1:2:2). Then all pots were autoclaved at the pressure of 21psi for at least 20 minutes at $121^0$C. Pumpkin var. Nutan was selected as a test plant for the experiment. About 3-5 seeds of pumpkin were directly shown in each pot on April 05, 2018. After the germination of seeds, thinning was done and a healthy one was kept in each pot.

**Preparation of pure culture and inoculation of root-knot nematode (*M. incognita*)**

Infected brinjal roots were collected from the field. A single eggmass was put in a petridish and same female from the gall was taken out on slide for the identification by the cutting of perennial pattern (Hartman and Sasser, 1985). After confirmation of *M. incognita*, the same egg mass of petridish was inoculated in tomato (*Solanum lycopersicum* L.) seedling for multiplication. After 45 days, subculturing was done to obtain sufficient amount of eggmasses. For the experiment roots were uprooted and washed free of soil and cut into 2 cm pieces. The sodium hypochlorite procedure was applied for extraction of eggs. The eggs were allowed to hatch into distilled water. After 24 h, J2s were collected and used in the experiments. Four leaves stage plants were inoculated with 2000 $J_2$.

**Simulated acid rain (SAR) preparation and application**

SAR was prepared by adding mixture of nitric acid ($HNO_3$) and sulfuric acid ($H_2SO_4$) into 1:3 ratio to deionize distilled water. The values of the SAR were adjusted to maintain different pH (6.0, 5.5, 5.0, 4.5, 4.0, 3.5 and 3.0.) levels by pH meter and sprayed freshly each time to plants twice in a week for two months.

**Termination and Parameters**

**Plant growth and yield:** The experiment was terminated after 60 days of nematode inoculation. Plant length, fresh and dry weights of shoot and root were determined. Shoot length was measured from the point of emergence of the first root to the shoot apex and cut from this point and root length was also taken. Fresh weight of shoots and roots were taken and kept in a hot air oven at 60 °C for 48 h for dry weight. Yield was determined as the number of flowers (male and female), number of fruits/plant, size of fruits (length and diameter) and weight of fruits (fresh and dry) per plant.

**Leaf area:** Leaf area was determined by taking five average sized of leaves from each treatment. Each leaf was placed on a 1 $mm^2$ graph paper. The leaf size was traced on the paper and the total area was calculated based on the number of squares covered within the traced region. The formula for leaf area estimation was followed as below:

A = KLB ($cm^2$), thus: K =A/LB

Where: A = Leaf area, L = Leaf length, B = Leaf width, K = Correlation coefficient which is constant

**Estimation of chlorophyll and carotenoids:** Photosynthetic pigments were estimated by Mac Lachlan and Zalik (1963) method. It was calculated according to the formulae given below.

i) Chl a = 12.7(O.D. 663) – 2.69(O.D.645) × V/1000×W (mg/g)

ii) Chl b =22.9(O.D. 645) – 4.68(O.D.663) ×V/1000 ×W (mg/g)

iii) Total Chl(a+ b) = 20.2(O.D.645) – 8.02(O.D.663) ×V/1000×W (mg/g)

iv) Carotenoids = 7.6(O.D.480) – 1.49(O.D.510) / D×1000×W (mg/g)

**Carbohydrate and protein estimation**: Estimation of carbohydrate was done by 'Anthrone' method (Hedge and Hofreiter 1962). The quantitative protein was estimated by Lowry et al. (1951) method.

**Estimation of NPK:** For the estimation of nitrogen and phosphorus in leaves, well known methods of Linder (1944) and Fiske and Subba Rao (1925) were employed repectively, while potassium was done by flame photometer.

**Relative water content (RWC):** RWC represents a useful indicator of the state of water balance of a plant, essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation (Gonzalez and Gonzalez-Vilar, 2001). Formula enunciated by Slatyer in 1967 was used to determine the RWC in leaves.

RWC = (FW-DW)/(TW-DW)X100

Where: FW= fresh weight, DW= dry weight, TW= turgid weight

**Leaf epidermal character:** The freshly mature leaves from the unharvested plants at the end of the exposure were collected. The leaves pieces were cut into 1 cm size. For study of upper epidermis, lower epidermis was damaged by gentle scrapping. Similarly, for lower epidermis, upper epidermis was scrapped. Now pieces were washed thrice with water then pieces were boiled in 20% HNO3 for 2-3 minutes to separate the epidermal peels separately. The peelings were washed thrice with water and transferred to 20% KOH for 15 min. for neutralization. Again peelings were washed thrice. After washing, the peelings were ready for staining (Ghause and Yunus, 1972). The washed peelings were kept in freshly prepared iron alum (1%) for 2-3 minutes, then washed thrice and stained in haematoxylin for 1-2 minutes, again washed 3-5 times and transferred to alcohol series (30, 50, 70 and 90%)) and then finally in absolute alcohol + xylene and xylene. The peelings were mounted in Canada balsam. The slides were examined under light microscope. The number of stomata, size (length and width in µm), size of stomatal aperture (length and width in µm) and trichomes (number in $mm^{-3}$ and length in µm) on lower and upper surfaces of the leaf were counted and calculated in 1mm" surface.

**Galls, egg masses and gall index:** The root of each plant was washed under tap water and immersed in an aqueous solution of phloxin B (0.15 g/liter tap water) for 15 minutes to stain the egg masses. Then galls and egg masses per root system were counted. For gall index, scale of Taylor and Sasser (1978) was followed. For reproduction factor ($R_f$) following formula was used:

$R_f$ = Fp/Ip

Where, Fp= final population and Ip= initial population

**Statistical Analysis:** Data obtained were subjected to Duncan Multiple Range Test (DMRT) and Least Significance Difference (LSD) at P=0.05 using R, Agricolae Package [R, 2.14.1].

## RESULTS

**Growth and yield:** The data summarized in table 1 and 2 indicate that all doses of simulated acid rain (SAR) and nematode together were harmful to pumpkin crop. All plant growth like length (shoot and root), fresh and dry weight (shoot and root) and yield (No. of flower and fruits, length, diameter and weight of fruits) were significantly reduced (P=0.05) in all treatments compared to control. Reductions were directly proportional to the acidity levels (inoculated with nematodes). Greater reduction in growth and yield were observed at pH 3.0 + N treatment.

**Leaf symptoms and photosynthetic pigments:** SAR-treatments of pH 3.0, 3.5, 4.0 and 4.5 caused white-to-tan irregular lesions on both the abaxial and adaxial surfaces of pumpkin leaves. The foliar symptoms were more pronounced in SAR-treatment at the highest pH 3.0, while the chlorosis, early leaf senescence, necrosis, leaf abscission, leaf folding and death were less pronounced in SAR at pH 5.0, 5.5 and 6.0. The foliar injury symptoms began to appear earlier (after two weeks of exposure) at pH 3.0, while at pH 3.5 4.0 and 4.5 in later stage (3 and 4 weeks). Chlorophyll a, b and carotenoids were significantly reduced by SAR-treatment (P= 0.05) relative to pH 3.0, 3.5, 4.0 and 4.5 before and after flowering. Greater reduction in the pigment synthesis was caused by SAR solution of pH 3.0 compared to both control inoculated and (unioculated) while no significant difference in chlorophyll a, b and carotenoids content was recorded at pH 6.0, 5.5 and 5.0 before and after flowering (Table 3). Moreover, Chlorophyll a was more affected than chlorophyll b.

**Table 1. Interactive effect of different levels of simulated acid rain and root-knot nematode *(M. incognita)* on growth of pumpkin var. Nutan**

| Treatments (SAR + S+ N) | Length (cm) | | | Fresh weight (gm) | | | Dry weight (gm) | | | No. of leaves | Average leaf area ($cm^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | | |
| Control (S) | 290a | 62a | 352a | 220.4a | 60.4a | 280.8a | 30.2a | 8.1a | 38.3a | 32a | 44a |
| Control (N) | 275ab | 52b | 327bb | 198.1b | 49.6b | 247.7b | 23.0b | 5.3b | 28.3b | 26b | 36b |
| 6.0 + N | 273b | 50bc | 323bc | 196.4b | 47.6b | 244.0bc | 22.2b | 5.1b | 27.3b | 24b | 34b |
| 5.5+ N | 272bc | 49bcd | 321cd | 195.2bc | 46.4bc | 241.6bc | 21.1bc | 4.8bc | 25.9bc | 23bc | 33b |
| 5.0+ N | 270bc | 47bcde | 317de | 193.1bcd | 44.2bc | 237.3cd | 19.2bc | 4.5cd | 23.7bcd | 21bc | 31bc |
| 4.5+ N | 262bcd | 43cdef | 305ef | 189.0cde | 41.0cd | 230.0de | 17.0bcd | 3.5de | 20.5cd | 20bcd | 30bc |
| 4.0+ N | 258bcd | 42def | 300fg | 186.4de | 39.6cd | 226.0e | 15.3cd | 2.9ef | 18.2de | 17cd | 26bc |
| 3.5+ N | 252cd | 39ef | 291g | 185.2ef | 35.6d | 220.8e | 12.2de | 2.2fg | 14.4ef | 14de | 21de |
| 3.0+ N | 246d | 36f | 282h | 179.5f | 29.1e | 208.6f | 9.5e | 1.6g | 11.1f | 10e | 17e |

Each value is a mean of five replicates, SAR- Simulated acid rain, S-Soil, N- Nematode

a, b, c Means with different superscripts in the same row are statistically different (P<0.05) according to Least Significant Test (LSD).

**Protein, carbohydrate, NPK and leaf relative water content:** The data presented in table 4 shows that all biochemical parameters like protein, carbohydrate, NPK and leaf relative water content were significantly (P= 0.05) reduced by the exposure of SAR treatment at pH 3.0, 3.5, 4.0 and 4.5. More reduction in the biochemical parameters were found at pH 3.0 of SAR exposure compared to both control (inoculated and unioculated) while no significant difference were recorded in all above biochemical parameters at the levels of chlorophyll at pH 6.0, 5.5 and 5.0.

**Table 2. Interactive effect of different levels of simulated acid rain and root-knot nematode *(M. incognita)* on yield of pumpkin var. Nutan.**

| Treatments (SAR + S + N) | No. of flower | | No. of fruits | Fruit length (cm) | Fruit diameter (cm) | Fruit weight (gm) | |
|---|---|---|---|---|---|---|---|
| | Male | Female | | | | Fresh | Dry |
| Control (S) | 22a | 23a | 22a | 14.0a | 8.5a | 3.25a | 1.98a |
| Control (N) | 19ab | 18ab | 16ab | 12.0ab | 6.5b | 2.60ab | 0.95ab |
| 6.0 + N | 18ab | 17ab | 15ab | 12.3ab | 6.1b | 2.55ab | 0.82bc |
| 5.5 + N | 16bc | 14bc | 13cd | 10.2bc | 5.6b | 2.50b | 0.78bc |
| 5.0 + N | 15bcd | 12cd | 10d | 8.5cd | 5.2b | 2.36b | 0.74d |
| 4.5 + N | 13cde | 11cd | 08de | 6.7de | 4.7d | 2.33b | 0.63de |
| 4.0 + N | 11de | 10de | 06def | 5.2e | 4.2e | 2.20bc | 0.57e |
| 3.5 + N | 09ef | 08def | 04fg | 5.9e | 3.9ef | 1.50cd | 0.52ef |
| 3.0 + N | 06f | 05fg | 02h | 4.5e | 3.5f | 1.30d | 0.45fg |

Each value is a mean of five replicates, SAR- Simulated acid rain, S- Soil, N- Nematode

a, b, c Means with different superscripts in the same row statistically different (P=0.05) according to Least Significant Test (LSD).

**Leaf epidermal character:** The data given in table 5 show that reduction in number, length and width of stomata, length of stomatal aperture of both surfaces were directly proportional to acidity (inoculated with nematode). While width of stomatal aperture was widen with the increasing acidity levels. As acidity level was increased, aperture width of both surfaces were also significantly (P = 0.05) increased as compared to control. Highest reduction was observed in pH 3.0 of acidity level. Surprisingly, trichomes number and length of both surfaces were significantly increased in lower acidity levels (6.0, 5.5, 5.0, 4.5 pH) and significantly decreased at higher acidity levels (4.0, 3.5 and 3.0 pH).

**Table 3. Interactive effect of different levels of simulated acid rain and root-knot nematode *(M. incognita*) on photosynthetic pigments of pumpkin var. Nutan.**

| Treatments (SAR + S + N) | Photosynthetic pigments (mg.g-1 fresh leaf) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Before flowering | | | | After flowering | | | |
| | Chl. a | Chl. b | Total chl. | Carotenoids | Chl. a | Chl. b | Total chl. | Carotenoids |
| Control (S) | 0.82a | 1.44a | 2.24a | 0.78a | 0.33a | 0.35a | 0.68a | 0.21a |
| Control (N) | 0.61b | 1.25b | 1.85b | 0.61b | 0.22b | 0.24b | 0.46b | 0.18ab |
| 6.0 + N | 0.59b | 1.23bc | 1.82bc | 0.60b | 0.20bc | 0.23b | 0.43bc | 0.16bc |
| 5.5 + N | 0.57b | 1.21bcd | 1.78cd | 0.57bc | 0.19bcd | 0.21bc | 0.40c | 0.15bc |
| 5.0 + N | 0.56bc | 1.19cde | 1.76d | 0.53cd | 0.17cd | 0.18cd | 0.35d | 0.13cd |
| 4.5 + N | 0.52cd | 1.17de | 1.69e | 0.51d | 0.15de | 0.17cd | 0.32d | 0.10de |
| 4.0 + N | 0.48de | 1.14ef | 1.62f | 0.48de | 0.11ef | 0.15de | 0.26e | 0.08ef |
| 3.5 + N | 0.45e | 1.10fg | 1.55g | 0.45ef | 0.09fg | 0.12ef | 0.21f | 0.06ef |
| 3.0 + N | 0.39f | 1.06g | 1.45h | 0.40f | 0.06g | 0.10f | 0.16g | 0.04f |

Each value is a mean of five replicates, SAR- Simulated acid rain, S-Soil, N- Nematode
a, b, c Means with different superscripts in the same row statistically different (P=0.05) according to Least Significant Test (LSD).

**Table 4. Interactive effect of different levels of simulated acid rain and root-knot nematode *(M. incognita*) on biochemical substances of pumpkin var. Nutan**

| Treatments (SAR + S + N) | Protein (µg fresh weight) | Carbohydrate (µg fresh weight) | Growth fertilizers (%) | | | Leaf relative water content (LWC %) |
|---|---|---|---|---|---|---|
| | | | Nitrogen (N) | Posphorus (P) | Potassium (K) | |
| Control (S) | 5.60a | 12.15a | 1.62a | 0.24a | 2.65a | 76.11a |
| Control (N) | 4.45b | 10.12b | 1.45b | 0.20ab | 2.40b | 55.33b |
| 6.0 + N | 4.25c | 10.07b | 1.39c | 0.18bc | 2.35c | 52.21c |
| 5.5 + N | 4.16cd | 9.95c | 1.31d | 0.15cd | 2.28d | 48.43d |
| 5.0 + N | 4.05de | 9.55d | 1.26e | 0.14cd | 2.19e | 46.11e |
| 4.5 + N | 3.95ef | 8.99e | 1.21f | 0.12de | 2.10f | 43.89f |
| 4.0 + N | 3.89ef | 8.19f | 1.17f | 0.11de | 2.01g | 40.45g |
| 3.5 + N | 3.80fg | 7.91g | 1.12g | 0.09ef | 1.95h | 37.65h |
| 3.0 + N | 3.72g | 6.99h | 1.05h | 0.06f | 1.89i | 33.99i |

Each value is a mean of five replicates, SAR- Simulated acid rain, S- Soil, N- Nematode
a, b, c Means with different superscripts in the same row statistically different (P=0.05) according to Least Significant Test (LSD).

**Galls, egg masses, gall index, eggmass index and reproduction factor**: The data given in table 6 show gall and eggmass per plant, reproduction factor and disease intensity in terms of gall and eggmass indices were significantly (P= 0.05) reduced by the exposure of SAR treatments at pH 6.0, 5.5 and 5.0 while none of the galls and eggmass were observed in subsequent higher treatments (4.5, 4.0, 3.5 and 3.0 pH).

**Table 5. Interactive effect of different levels of simulated acid rain and root-knot nematode *(M. incognita)* on leaf epidermal character of pumpkin var. Nutan**

| Treatments (SAR + S + N) | Leaf surface | Stomata | | | | | Trichomes | |
|---|---|---|---|---|---|---|---|---|
| | | Number ($mm^{-3}$) | Size of stomata (µm) | | Size of stomatal aperture (µm) | | Number ($mm^{-3}$) | Length (µm) |
| | | | Length | Width | Length | Width | | |
| Control (S) | Abaxial | 20.21 | 3.44 | 2.15 | 2.33 | 1.15 | 6.20 | 732.73 |
| | Adaxial | 15.65 | 3.18 | 2.06 | 2.10 | 1.09 | 4.65 | 687.11 |
| Control (N) | Abaxial | 16.19 | 2.66 | 1.82 | 1.95 | 0.89 | 4.56 | 533.33 |
| | Adaxial | 17.11 | 2.50 | 1.65 | 1.98 | 0.98 | 3.15 | 565.44 |
| 6.0 + N | Abaxial | 15.05 | 2.45 | 1.70 | 1.87 | 0.92 | 4.38 | 524.09 |
| | Adaxial | 16.12 | 2.29 | 1.50 | 1.90 | 0.99 | 3.05 | 545.10 |
| 5.5 + N | Abaxial | 13.01 | 2.20 | 1.59 | 1.74 | 0.94 | 4.21 | 505.70 |
| | Adaxial | 14.99 | 2.14 | 1.40 | 1.82 | 1.02 | 2.93 | 525.10 |
| 5.0 + N | Abaxial | 11.41 | 1.56 | 1.38 | 1.60 | 0.96 | 3.90 | 490.16 |
| | Adaxial | 12.50 | 1.89 | 1.23 | 1.69 | 1.05 | 2.65 | 501.34 |
| 4.5 + N | Abaxial | 09.29 | 1.35 | 1.25 | 1.48 | 0.99 | 3.72 | 473.19 |
| | Adaxial | 10.88 | 1.70 | 1.14 | 1.48 | 1.06 | 2.54 | 485.91 |
| 4.0 + N | Abaxial | 07.28 | 1.20 | 1.13 | 1.24 | 1.05 | 3.45 | 452.22 |
| | Adaxial | 09.67 | 1.58 | 1.02 | 1.26 | 1.08 | 2.36 | 461.76 |
| 3.5 + N | Abaxial | 05.76 | 0.99 | 1.05 | 1.10 | 1.10 | 3.08 | 402.65 |
| | Adaxial | 07.88 | 1.30 | 0.87 | 1.11 | 1.10 | 2.17 | 440.10 |
| 3.0 + N | Abaxial | 03.66 | 0.68 | 0.80 | 0.93 | 1.19 | 2.76 | 385.89 |
| | Adaxial | 05.29 | 0.96 | 0.75 | 0.95 | 1.13 | 1.91 | 390.20 |

Each value is a mean of five replicates, SAR- Simulated acid rain, S- Soil, N- Nematode

**Table 6. Effect of different levels of Simulated acid rain on root-knot disease caused by of *M. incognita* in pumkin var. Nutan.**

| Treatments (SAR + S + N) | Number/root sysytem | | Number of eggs/egg mass | Reproduction factor (Rf) | Gall index | Eggmass index |
|---|---|---|---|---|---|---|
| | Galls | Egg masses | | | | |
| Control (N) | 230 | 155 | 215 | 24.72 | 5 | 5 |
| 6.0 + N | 80 | 20 | 173 | 6.92 | 4 | 3 |
| 5.5 + N | 10 | 02 | 131 | 0.65 | 2 | 1 |
| 5.0 + N | 02 | 00 | 79 | 0.19 | 1 | 0 |
| 4.5 + N | 00 | 00 | 00 | 00 | 0 | 0 |
| 4.0 + N | 00 | 00 | 00 | 00 | 0 | 0 |
| 3.5 + N | 00 | 00 | 00 | 00 | 0 | 0 |
| 3.0 + N | 00 | 00 | 00 | 00 | 0 | 0 |

Each value is a mean of five replicates, SAR- Simulated acid rain, S- Soil, N-Nematode

## DISCUSSION

Acid rain causes morphologically and anatomically injury to plants (Wood and Bormann, 1974), producing symptoms as chlorosis and necrosis in leaves (Shriner, 1978). The biotic plant pathogens are also affected by the acidity (Asai and Futai, 2005; Khan and Khan, 1994; Singh, 1989). Chlorosis, necrotic lesions and tip injury on leaves of pumpkin were observed in plants exposed to SAR.

Symptoms were more pronounced in pH 3.0 and no any chlorosis, necrotic lesions and tip injury were observed at the concentrations of 6.0, 5.5 and 5.0 pH. Similar results were also observed by Johnston and Shriner (1985) on leaf tip necrosis in wheat at pH 4.3 and 2.3. While, no foliar injury was observed in wheat plants exposed to pH 5.0 and 4.0 levels. It was due to low sensitivity of pumpkin plants to lower acid rain levels based on visible effect of foliage. The SAR also suppressed the influence of *M. incognita* in all the combinations on pumpkin crop. That is why combined impacts of both irrespective on plant growth, yield, photosynthetic pigments, carbohydrate and protein, NPK, leaf water contents and leaf epidermal characters were harmful with increasing the acidity levels. However, magnitude of effects and amount of suppression varied. Lee et al. (1980) reported that even if visible injuries did not develop under SAR conditions, reduction in plant growth could be detected. Shriner and Johnston (1981) have also concluded that pH level was mainly responsible for growth reductions of soybean caused by acid rain. The reports available in literature show that reduction in growth and yield of crop plants generally occurs when plants are exposed to acidified rain (Evans et al., 1986; Johnston and Shriner, 1985; Singh, 1989). Similarly, SAR also hampered to all biochemical parameters of wheat. Photosynthetic pigments were inhibited with respect to acidity levels (Kausar and Khan, 2010). Reduction in the pigments was perhaps due to removal of $Mg^+$ from the tetrapyrol ring of chlorophyll molecule by $H^+$ (Foster, 1990) or due to the increase in transpiration rate by acid rain (Evans et al., 1977). Similar results were observed on alfalfa, lentil, chickpea and tomato leaves (Khan and Khan, 1994; Takemoto et al., 1988; Singh, 1989). Recently, reduction in photosynthetic pigments have also been observed in many crops i.e. mustard, radish, potato and *Phaseolus vulgaris* (Agrawal et al.,2005; Kausar et al., 2005; Khan and Devpura, 2005; Varshney et al., 2005). Kausar and Khan (2009) documented that at pH 4.0 and 3.0 seed carbohydrate and seed protein of wheat plant were suppressed by simulated acid rain. The reduction in carbohydrate contents might have been caused through the overall poor growth leading to poor yield (seeds). Proteins were also reduced in leaf of pumpkin exposed to SAR in the present study. Such an effect could be due to inhibition of amino acid under acidic condition, which is a constituent of protein synthesis. Reduction in protein contents has also been observed by acid rain in chickpea, lentil, soybean, wheat etc. (Evans et al., 1983; Khan and Devpura, 2005; Singh, 1989). Surprisingly, the width aperture of stomata was increased. Foster (1990) recorded appreciable losses of $K^+$, $Ca^{++}$ and $Mg^{2+}$ from the foliage of tomatoes exposed to acid rain at pH 5.6-2.5. These losses might have been responsible for the wide aperture of stomata of wheat plants treated with SAR. The trichomes number and length was increased in plants exposed to pH 5.0. It may be due to adaptive response induced in plants to provide mechanical defense against pollutant (Levin, 1973). Similar result was observed on tomato at pH 5.6 by Khan and Khan (1994) and on the wheat at pH 3.0 (Kausar and Khan, 2010).

Bolla and Fitzsimmons (1988) found that reproduction *of Bursaphelechus xylophilus* was decreased in pine seedlings treated with acid rain. Singh (1989) observed reduced gall formation and reproduction of M *incognita* and *M. javanica* on chickpea and lentil treated with acid rain. Khan and Khan (1994) observed antagonistic interaction between SAR and M. *incognita* on tomato. The present findings are in accordance to these observations. Some infections were observed at lower level of SAR (6.0. 5.5 and 5.0 pH). This might be due to escape of some larvae from the influence of SAR, which succeeded in developing disease. Galls formation occurred in these plants. At higher levels of pH (4.5, 4.0, 3.5 and 3.0) the none of the galls and eggmasses were formed. Thus result showed that there was antagonistic interaction between SAR and root-knot nematode.

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