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Morphological, Physiological and Biochemical Changes in Characters of Brassica nigra L. Affected by Foliar Application of Salicylic Acid

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Abstract

A field experiment was conducted to investigate the morphological, physiological and biochemical Changes in brassica plant affected of salicylic acid in the department of botany, Gujarat University. Plants produce some specific chemical substances, which are capable of moving from one organ to another and provide physiological control on growth. These substances, which are active in very small amount are called plant hormones or growth regulators. Salicylic acid is well known phytohormone, emerging recently as a new paradigm of an array of manifestations of growth regulators. As a signal molecule, it plays an essential role in regulating many physiological processes of plants, including flowering, seed germination, heat production, membrane permeability, defence responses, etc.The level of SA varies from ng to mg per gram of fresh weight in different plant species and it will be changed under biotic and abiotic stresses for enhancing plants stress tolerance. The experiment consisted of four different concentartion (control, 10 ppm, 20 ppm, 30 ppm). The effect was checked during 5 days of interval till 20 days the effect will noted. Mustrad plant is exposed to all parameters like growth, development, morphological, biochemical, physiological and levels of chlorophyll. Plant significantly responses to all the parameters and higher response in concentration of 20ppm during 10 days of interval. When we treat plant with SA, plant becomes protected against the drought stress, cold stress also. SA is important for plant growth and development, defence response, germination, flowering, medicinally, etc.

Key words:*Brassica nigra L.* (Mustrad), plant growth regulators; salicylic acid SA; foliar application; growth characteristics; biochemical constituents.

Introducton:

Brassica nigra the black mustard is an annual plant cultivated for its black or brown seeds, at which are commonly used as spice. It is originated from tropical regions of North Africa, temperate regions of Europe and parts of Asia (north temperate region). According to Dutt and chopra (2001) Indian mustard was grown in season September to octormber. It is harvested in February to march. Indian mustard is cultivated in the state of Assam, Bihar, Gujarat, etc. It prefers soil well aerated soil that does not become water logged. Mustard performs best in the soil with a netural (pH 7) but will tolerate alkaline above 7 and slight saline soils. The crop was grown in

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heavy rainfall 25-40 cm per year (hrideek T K, *et al*., 2004). It is an upright plant with large staked leaves. They are coverd with hairs or bristles at the base, but on the stem smoother. It blooms in summer, from May onwards. Each stem has four flowers at the top, forming a ring around the stem. Mustard plants are annual, biannual or perennial herbs in some species.

Indian mustard is the most important winter oilseed crop. It is generally grown under rain fed conditions and moderately tolerant to soil acidity, it requires well drained soil having pH near to neutral. Oil content in seeds varies from 37 to 49 per cent. The seed and oil of mustard have a peculiar pungency due to presence of glucosinolate and its hydrolysis products such as allyl-isothiocynate making it suitable to use as condiment in the preparation of pickles and for flavoring curries and vegetables. Its oil mainly used in cosmetics, medicines and various other industries. Viewed in global context, India occupies premier position in global oilseed scenario, accounting for about 23.5 per cent area and 17.6 per cent of production (FAO, Year book, 2010). Among the seven edible oilseeds cultivated in India, rapeseed-mustard contributes 28.6 per cent in the total oilseeds production and ranks second after groundnut sharing 27.8 per cent in the India's oilseed economy (Shekhawat, *et al.,* 2012). The rapeseed-mustard group broadly includes Indian mustard, yellow sarson, brown sarson, raya, and toria crops.

Oilseed constitutes the second largest agricultural commodity after cereals in India, producing 11.94 mt from 22.9 m ha area with the average productivity of 955 kg ha-1 and 6.4 mt production from 5.5 m ha area for rapeseed and mustard with the average productivity of 1159 kg ha-1 accounting 22.7 per cent in area and 25.9 per cent in production in oilseed scenario (Economic Survey, 2011).

Plant hormones are a group of naturally occurring, organic substance which influence physiological processes at low concentrations. The processes influences consist mainly of growth, differentiation and development, through other processes, such as stomata movements, may also be affected. Plant hormones have also been refered to as "Phytohormones" though this term is seldom used (Peter j. Davies, 1987).

Phytohormones as an organic compounds produce naturally in higher plants, controlling growth or other physiological function as a site remote from its place of production and active in minute amount. The term phytohormones distinguish from animal hormones (Thimann, 1948).

The term "hormone" comes originally from the Greek and is used in animal physiology to denote a chemical messenger. Plant growth and development involves the integration of many environmental and endogenous signals that, together with the intrinsic genetic program, determine plant form. Fundamental to this process, several growth regulators are collectively known as the "plant hormones" or "phytohormones" (Pranav kumar and usha mina, 2017).

Plant hormones are small organic compounds, synthesized by specific plant cell/ tissues, active at low concentration and promote or inhibit growth and developmental processes. The concentrations required for plant are very low 10-6 to 10-5 mol/L. Plant hormones are the naturally occurring organic substances. Hormones are important for yield, improve the quality and facilitate harvesting. Plant hormones regulate cellular processes in targeted cells locally and

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moved to other locations, in other functional parts of the plant. Hormones determine the formation of the flower, stem, leaves, the shedding of leaves, the development and ripening of fruit.

Plant productivity is severely affected by abiotic stress factors viz., salinity, drought, high and low temperature, and heavy metals. Rapid and effective measures of plant treatments are necessary so that decline of crop due to high temperature can be controlled successfully. Out of the various abiotic stresses, high temperature is the second most important stress, which can strike crop at any time and impose many limitations on growth and development thermo tolerance using various genetic approaches can mitigate the adverse effects of heat stress. For this purpose, a thorough understanding of physiological responses of plants to high temperature, and possible strategies for improving crop thermo tolerance is imperative. The application of plant growth regulators is known to play an significant role in plant response to stress. Salicylic acid plays diverse physiological roles in plants which includes thermogenesis, flower induction, nutrient uptake, ethylene biosynthesis, stomatal movement, photosynthesis and autioxidative enzymes (Hayat, *et al*., 2007). Heat tolerance can be induced in a plant by prior exposure to moderately high temperatures which enables the plant to cope with subsequent potentially lethal, heat exposure (Howarth and Ougham, 1993). Salicylic acid has been found to be involved in both basal and acquired thermo tolerance in plants (Dat, *et al*., 1998a, b, 2000, Lopez Delgado, *et al*., 1998). Salicylic acid (SA), a plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development (Hayat, *et al*., 2010). . Under field conditions, high temperature stress is frequently associated with reduced water availability (Simoes-Araujo, *et al*., 2003). Shekhawat, *et al*., (2012) also found same result in Indian mustard. It has negative impact on physiology of plant and ultimately reduces the yield in all agricultural crops. Mustard crop is very sensitive to temperature stress during reproductive stage. In general, the negative impacts of abiotic stresses on agricultural productivity can be minimized by a combination of genetic improvement and cultural practices.

Material and methods:

A field experiment was carried out at the gujarat university (botanycal graden), during the successive seasons of 2018/2019 to study the effect of foliar application of Salicylic acid (SA), on vegetative growth, biochemical test and its component as well as photosynthetic pigments content in the leaves, total protein, total suger, reducing suger, protein, starch content of mustrad.

The experimental design was in the four rows of pots. Salicylic acid treatments occupy the main plots treatments were allocated at random in sub-plots. Seeds of mustrad were sowing in suitable time and let them grow till their pre plants are at four leaf stage. During growing season equal amount of water and environmental condition. When the plants are mature we started to give foliar effect of salicylic acid at equal amount in 5 days of interwal.Mustrad plants were foliar sprayed with salicylic acid , each at the concentration of 10ppm, 20ppm, 30ppm.

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Environmental condition

Equal amount of water was poured daily in each set of pots. Pots were placed in a botanical cage of botanical garden under normal environment condition which provides natural temperature, light and humidity.

Experiment design

Total 12 pots were taken for the experiment three pots for control three pots each for treatment of 10ppm, 20ppm, 30ppm of salicylic acid. In each pot total 5gm seeds are added for the experiment. Treatment was started when plants were in four leaf stage and approx 25 days old. Treatment was given in the form of foliar spray.

Treatment

Salicylic acid treatment was given to the plants grow in pots. The power form of SA was dissolved in distilled water according to concentration.

Duration of treatment

Plants were treated with salicylic acid for 30 days duration in which these were applied at an interval of every 6 days till three reading were obtained.

Data and season of grown plant

The treatment was given from 18 Jan, 2019 to 5 Feb, 2019 during winter season.

Collection of data

Data was collected at an interval of every six days and reading was obtained.

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Growth Parameters studied

To study the effect of hormones on plant growth several parameters had been studied which are as follow:

1. Root length.
2. Shoot length.
3. Leaf length.
4. Root weight.
5. Shoot weight.
6. Leaf weight.
7. Number of the leaves.

The Estimation of Metabolites:

It includes the estimation of following metabolites:

A) Total sugar and reducing sugar

B) Proteins

C) Starch

D) Chlorophyll

Standard methods were followed for estimation of metabolites mentioned is as:

Total sugar and reducing sugar:

100mg plant material was weighed and homogenate with 10ml 80% ethanol. It was centrifuged for 10 minutes. Supernatant 1 was collected; while 10ml 80% ethanol was added again to the residue, centrifuge it and supernatant 2 was mixed with supernatant1. Residue was discarded. To 1ml alcoholic aliquot, 1ml 1N H_2SO_4 was added and heated at 490 C in water bath for 30 minutes for hydrolysis of the mixture 1-2 drop of methyl red indicator was added. 1N NaOH was added drop wise for the neutralization (colour was changed from pink to yellow). 1ml Nelson Somogyi's reagent was added to it and the tube was kept in boiling water bath for 20 minutes. After cooling of the test tube, 1ml arsenomolybdate was added and final volume as made up to 20ml with distilled water. Optical density was noted at 540nm. Blank was prepared in the same manner. Determine the concentration of sugar using regression equation. The result was expressed as mg/gm plant material.

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Starch:

100mg plant material was weighed and homogenate with 10ml 80% ethanol. It was centrifuged for 10 minutes. Supernatant 1 was collected; while 10ml 80% ethanol was added again to the residue centrifuge it and supernatant 2 was mixed with supernatant 1 and removed. Residue was used for starch estimation. The residue was dissolved in 20ml 0.7% KOH and boiled for gelatinization for 40 minutes. It was centrifuged after cooling and 1ml aliquot (Supernatant), 0.5ml 20% acetic acid; 1ml citrate buffer (0.05M, PH 5.0) and 1ml I_2KI were added and incubated at room temperature for 10 minutes. Optical density was taken at600mm. Blank was prepared in the same manner. Determine the concentration using starch stalk solution as a standard. The result was expressed as mg/gm plant material.

Total proteins:

Grind 1gm plant material in 10ml, 0.1M phosphate buffer (PH 7.0) using mortar and pestle. Centrifuge the extract at 10,000 rpm for 15 minutes at 40 C. Use the supernatant as extract for estimation of total soluble proteins. Take 1ml extract and add 5ml Branford's reagent and mix well. Read the absorbance of the resultant solution which is a blue colored complex at 595nm. Determine the concentration of total soluble proteins using bovine serum albumin as a standard. The result was expressed as mg/gm plant material.

Total Chlorophyll:

Grind the plant material (500mg) in 10ml 80% acetone with sand in pestle and mortar. Centrifuge the extract at 10,000 rpm for 10 minutes. Use the supernatant for estimation of Chlorophyll content. Read the absorbance of the solution at 663nm, 652nm, and 645nm.

Result and discussion:

Fertilizer is most important for plant growth regulator. Fertilizer and plant related each other. When apply the hormone treatment on plant. The plant gives response their physiological and biochemical and morphological activity. Foliar spray is applied to plant. Water, environmental condition also affects the plant for growth and development. Plants are realted to all the biotic and abiotic condition.

This experiment actually showed the effect on plant growth of salicylic acid on mustrad plants.

Effect of fertilizer and Salicylic acid on plant growth.

The property of living things is that they respond to stimuli. Plants are living multicellular organism .which are considered as sensitive as humans for initial assaying of effect and testing new therapies.

The effect of the Salicylic acid on plant growth was studied on the given parameters.

1. Height of plant.

2. Number of leaves.

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3. Fresh weight of plant.

The result of this experiment is divided into two parts.

1. Fertilizer effect on the plant.

2. Biochemical analysis.

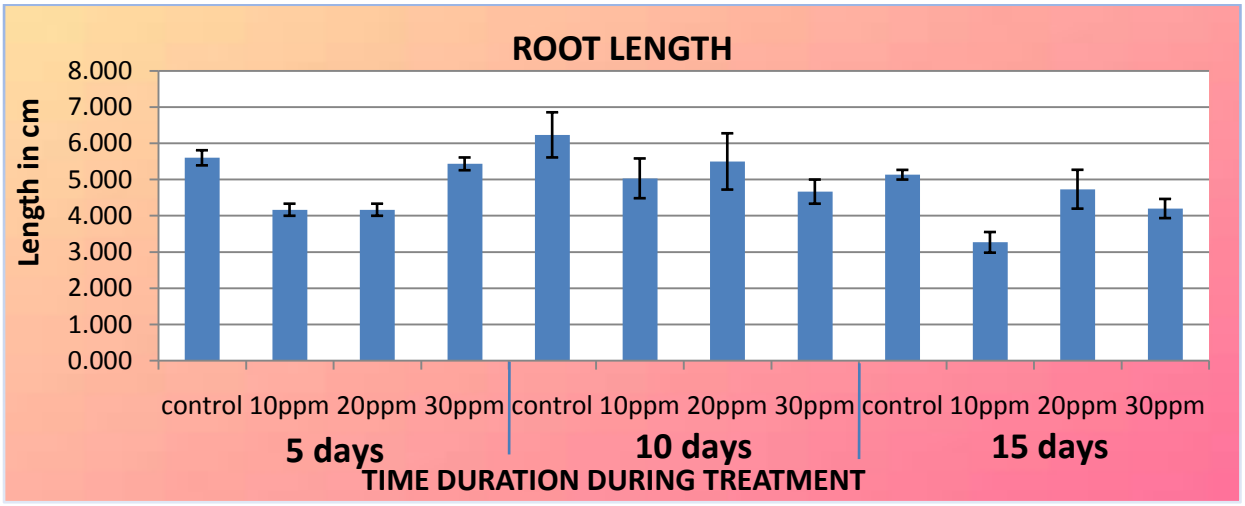
Length of the plants

Table 1: Root length of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Root Length (In cm)
Control	5	5.600±0.208
	10	6.233±0.623
	15	5.133±0.133
10 ppm	5	4.167±0.167
	10	5.033±0.549
	15	3.267±0.285
20 ppm	5	4.167±0.167
	10	5.500±0.777
	15	4.733±0.536
30 ppm	5	5.433±0.176
	10	4.667±0.333
	15	4.200±0.265

This table shows that the average length of the root was more in treated set as compared to growth of plants parts during 5 days of interval and compared to the control one according to time duration.

Fig. 1: Root length of the selected plant



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The root length was higher in concentration of 20 ppm after 10 days interval compared to others. In this study the root length was higher in concentration of 30 ppm after 5 days of interval, in 20 ppm concentration after 10 days of interval and in 20 ppm concentration after 15 days of interval. Generally the root length was high in interval of 10 days.

Table 2: Shoot length of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Shoot Length (In cm)
Control	5	10.833±0.441
	10	10.333±0.333
	15	9.200±0.153
10 ppm	5	9.600±0.586
	10	11.233±0.906
	15	10.933±1.049
20 ppm	5	9.100±0.985
	10	10.433±1.105
	15	10.933±0.809
30 ppm	5	9.233±0.433
	10	10.367±0.318
	15	10.967±0.524

This table shows that the average length of the shoot was more in treated set as compared to growth of plants parts during 5 days of interval and compared to the control one according to time duration.

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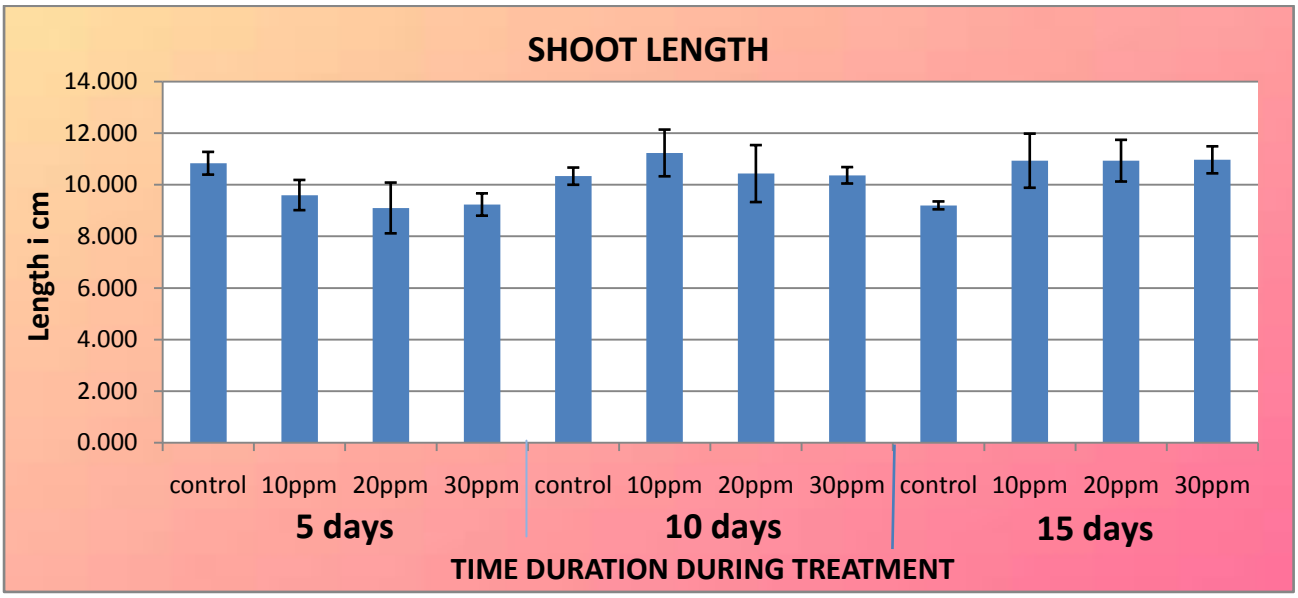
Fig. 2: shoot length of the selected plant



The shoot length was higher in concentration of 10 ppm after 10 days interval compared to others. In this study the shoot length was higher in concentration of 10 ppm after 5 days of interval, in 10 ppm concentration after 10 days of interval and in 30 ppm concentration after 15 days of interval. Generally the root length was high in interval of 15 days

Table 3: leaf length of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Leaf Length (In cm)
Control	5	1.100±0.115
	10	1.700±0.153
	15	1.567±0.033
10 ppm	5	1.033±0.240
	10	1.500±0.058
	15	1.400±0.208
20 ppm	5	1.367±0.120
	10	1.500±0.100
	15	1.700±0.115
30 ppm	5	1.167±0.088
	10	1.433±0.067
	15	1.467±0.067

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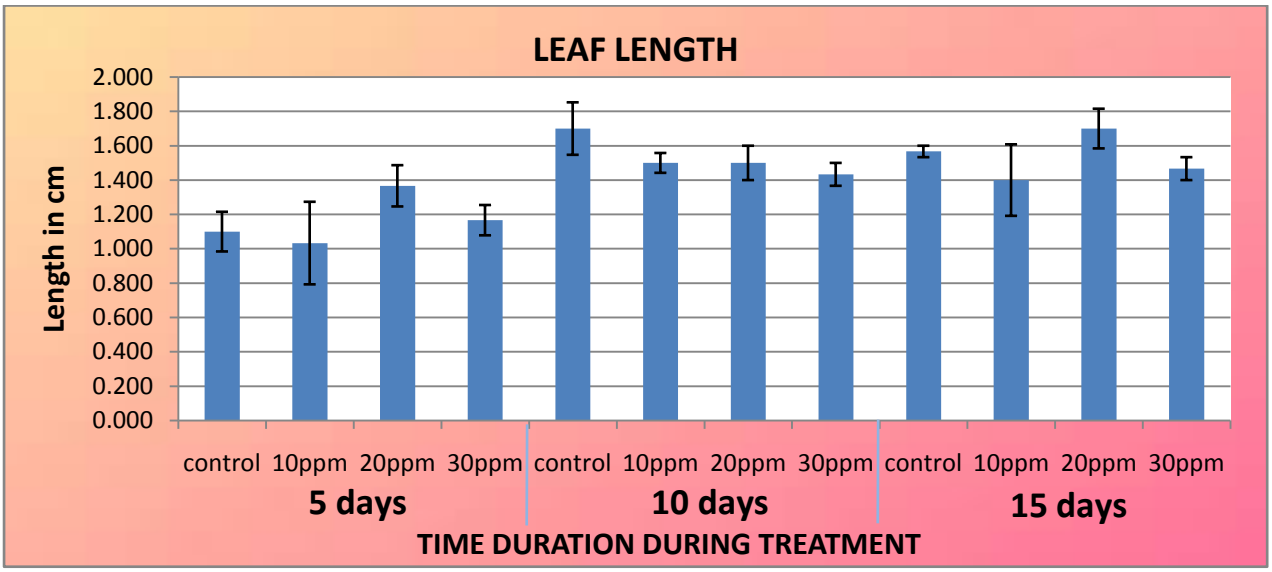
This table shows that the average length of the leaves was more in treated set as compared to growth of plants parts during 5 days of interval and compared to the control one according to time duration.

Fig. 3: leaf length of the selected plant



The leaf length was higher in concentration of 20 ppm after 15 days interval compared to others. In this study the leaf length was higher in concentration of 20 ppm after 5 days of interval, in 20 and 10 ppm concentration after 10 days of interval and in 20 ppm concentration after 15 days of interval. Generally the root length was high in interval of 15 days.

Weight of the plant

Table 4: Root weight of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Root Weight (In cm)
Control	5	1.800±0.404
	10	1.467±0.120
	15	1.433±0.033
10 ppm	5	1.200±0.200
	10	0.800±0.058
	15	1.600±0.289
20 ppm	5	1.367±0.467
	10	1.767±0.521
	15	0.800±0.058

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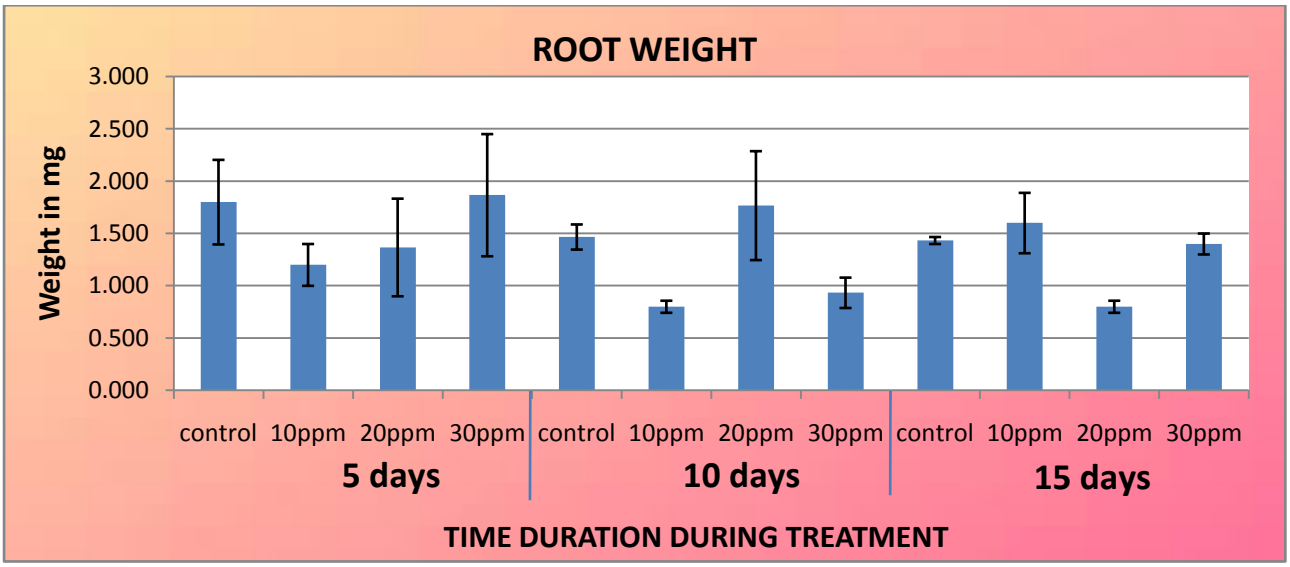
30 ppm	5	1.867±0.584
	10	0.933±0.145
	15	1.400±0.100

This table shows that the average weight of the root was more in treated set as compared to growth of plants parts during 5 days of interval and compared to the control one according to time duration.

Fig. 4: root weight of the selected plant



The root weight was higher in concentration of 30 ppm after 5 days interval compared to others. In this study the root weight was higher in concentration of 30 ppm after 5 days of interval, in 20 ppm concentration after 10 days of interval and in 10 ppm concentration after 15 days of interwval. Generally the root length was high in interval of 5 days.

Table 5: Shoot weight of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Shoot Weight (In cm)
Control	5	87.700±3.736
	10	126.233±12.397
	15	113.400±9.525
10 ppm	5	55.667±16.700
	10	142.700±17.970
	15	119.367±7.512
20 ppm	5	75.500±11.763
	10	108.533±5.612
	15	137.733±10.951

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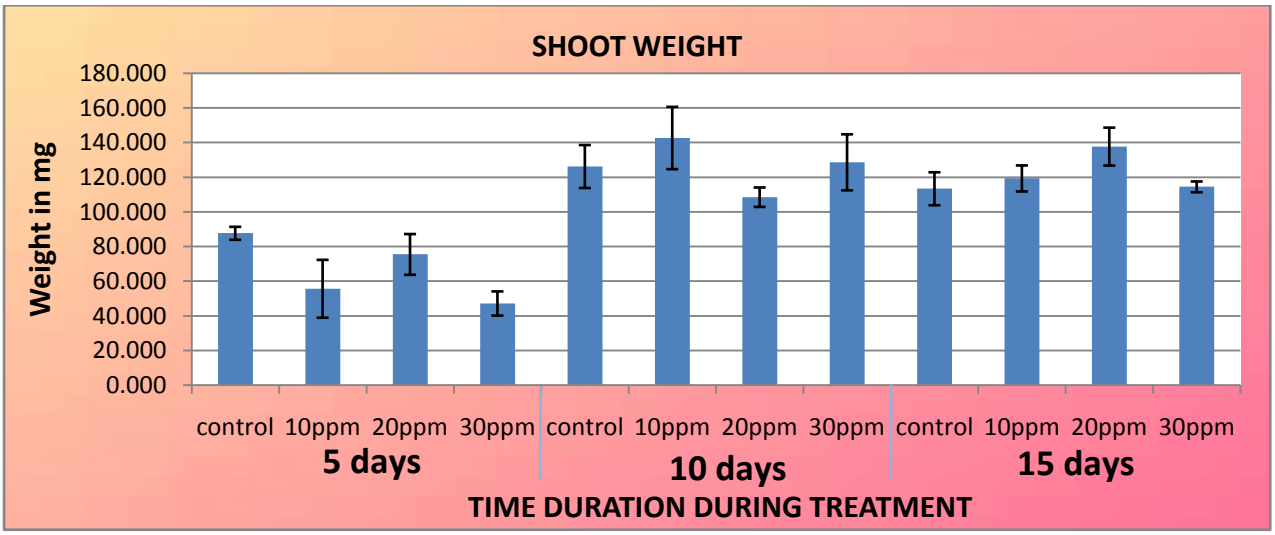
30 ppm	5	47.167±6.980
	10	128.667±16.192
	15	114.500±3.118

This table shows that the average weight of the shoot was more in treated set as compared to growth of plants parts during 5 days of interval and compared to the control one according to time duration.

Fig. 5: shoot weight of the selected plant



The shoot weight was higher in concentration of 10 ppm after 10 days interval compared to others. In this study the shoot weight was higher in concentration of 20 ppm after 5 days of interval, in 10 ppm concentration after 10 days of interval and in 20 ppm concentration after 15 days of interval. Generally the root length was high in interval of 10 days.

Table 6: Leaf weight of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Leaf Weight (In cm)
Control	5	54.467±5.922
	10	82.167±14.905
	15	87.033±9.298
10 ppm	5	36.100±6.393
	10	88.533±11.583
	15	67.733±10.203
20 ppm	5	65.500±10.768
	10	69.033±3.889
	15	75.133±12.541
30 ppm	5	32.800±3.584

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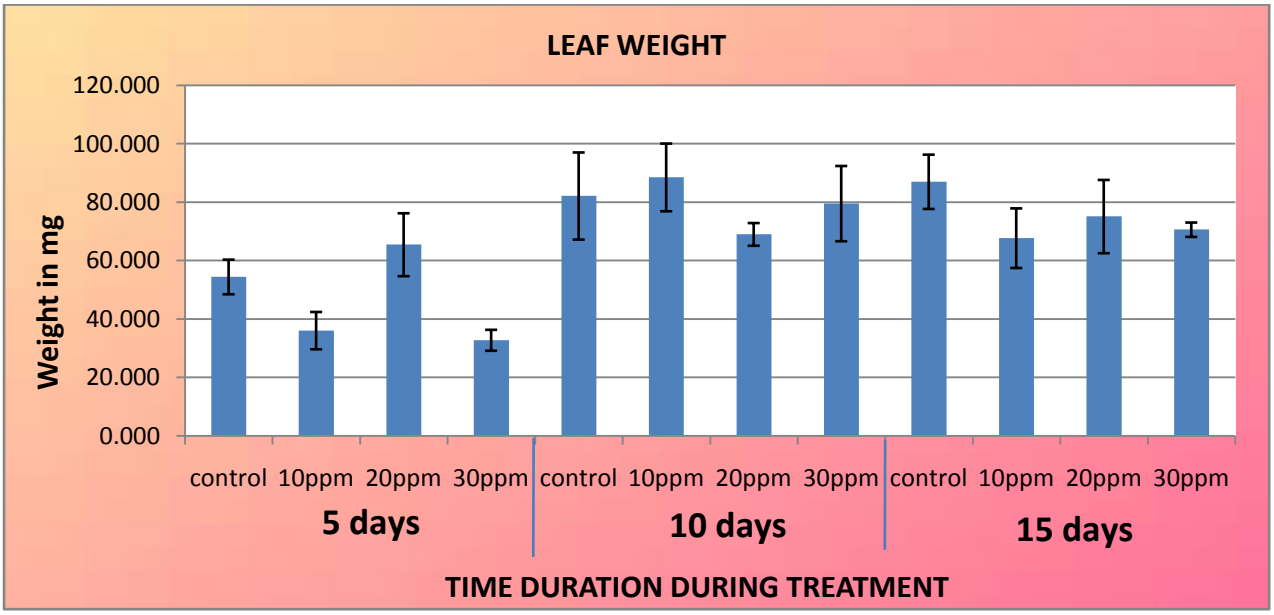
	10	79.567±12.879
	15	70.633±2.457

This table shows that the average weight of the leaves was more in treated set as compared to growth of plants parts during 5 days of interval and compared to the control one according to time duration.

Fig. 6: leaf weight of the selected plant



The leaf weight was higher in concentration of 10 ppm after 10 days interval compared to others. In this study the leaf weight was higher in concentration of 20 ppm after 5 days of interval, in 10 ppm concentration after 10 days of interval and in 20 ppm concentration after 15 days of interval. Generally the root length was high in interval of 10 days.

Table 7: Number of the Leaves of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	No Of Leaves (In cm)
Control	5	3.667±0.333
	10	4.667±0.667
	15	5.667±0.333
10 ppm	5	3.333±0.333
	10	5.000±0.577
	15	4.333±0.333
20 ppm	5	3.333±0.333
	10	5.333±0.333

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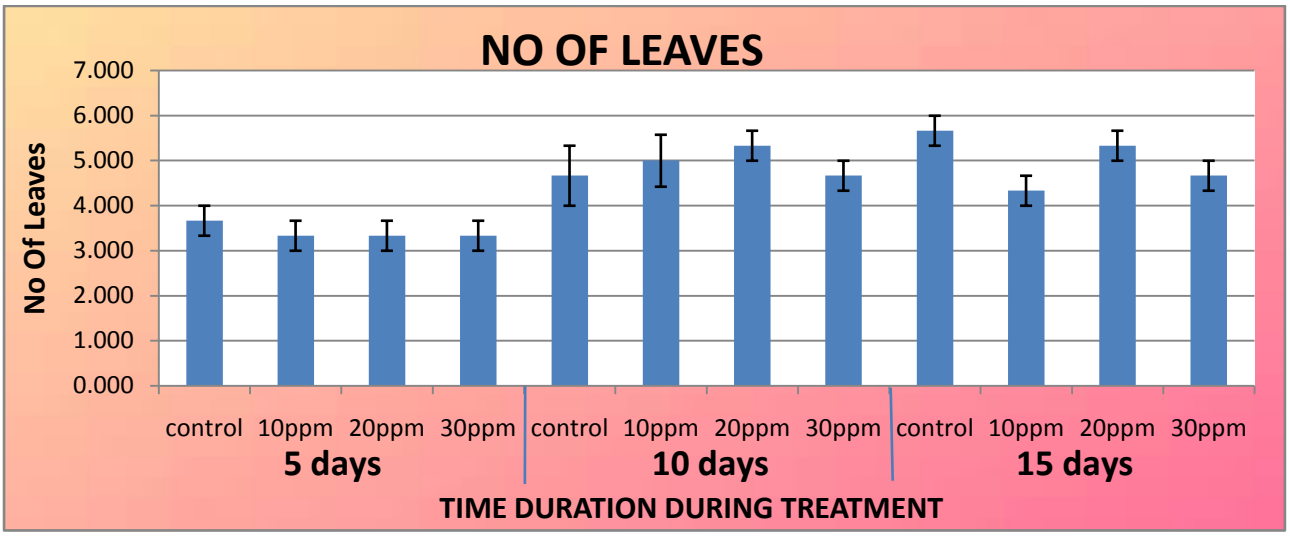
	15	5.333±0.333
30 ppm	5	3.333±0.333
	10	4.667±0.333
	15	4.667±0.333

This table shows that the average number of the leaves was more in treated set as compared to growth of plants parts during 5 days of interval and compared to the control one according to time duration.

Fig. 7: Number of the Leaves of the selected plant



The number of leaves was higher in concentration of 20 ppm after 10 days and 15 days interval compared to others. In this study the number of leaf was similar in all concentration after 5 days of interval, in 20 ppm concentration after 10 days of interval and in 20 ppm concentration after 15 days of interval. Generally the root length was high in interval of 10 and 15 days.

4.5 Result boichemical test

Table 8: Total sugar of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Total Sugar (mg/g)
Control	0.2	0.01408
	0.4	0.01408
	0.6	0.01409
	0.8	0.01409
	1	0.0141

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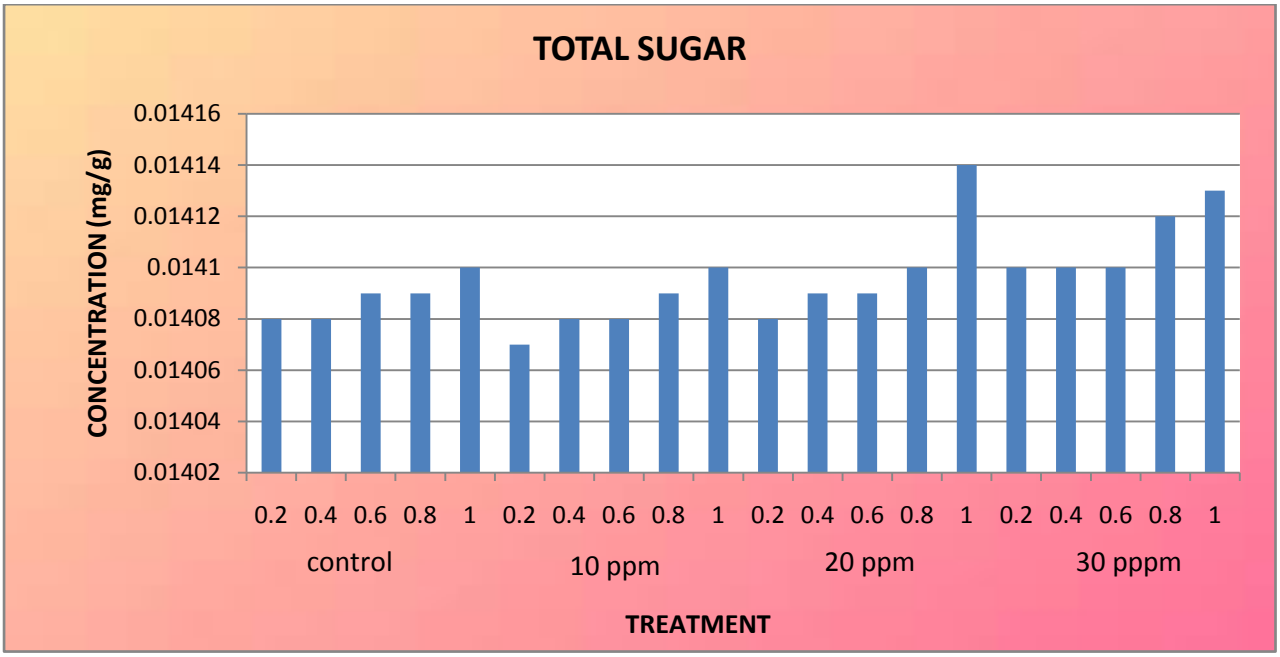
10 ppm	0.2	0.01407
	0.4	0.01408
	0.6	0.01408
	0.8	0.01409
	1	0.0141
20 ppm	0.2	0.01408
	0.4	0.01409
	0.6	0.01409
	0.8	0.0141
	1	0.01414
30 ppm	0.2	0.0141
	0.4	0.0141
	0.6	0.0141
	0.8	0.01412
	1	0.01413

This table shows higher concentration of total sugar compared to the control.

Fig. 8: Total sugar of the selected plant



This figure shows that the concentration of total suger with effect of salicylic acid. Concentration of total sugar was observed higher in 1 and 0.8 (mg/g) concentrations in all treated of plants and lower in 0.2 (mg/g) concentrations in 10 and 20 ppm as compared with control.

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Table 9: Reducing sugar of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Reducing Sugar (mg/g)
Control	0.2	0.046
	0.4	0.0462
	0.6	0.0463
	0.8	0.0465
	1	0.0466
10 ppm	0.2	0.0462
	0.4	0.0462
	0.6	0.0468
	0.8	0.0469
	1	0.0471
20 ppm	0.2	0.046
	0.4	0.0461
	0.6	0.0466
	0.8	0.0468
	1	0.0474
30 ppm	0.2	0.0463
	0.4	0.0464
	0.6	0.0465
	0.8	0.0471
	1	0.0473

This table shows higher concentration of reducing sugar compared to the control.

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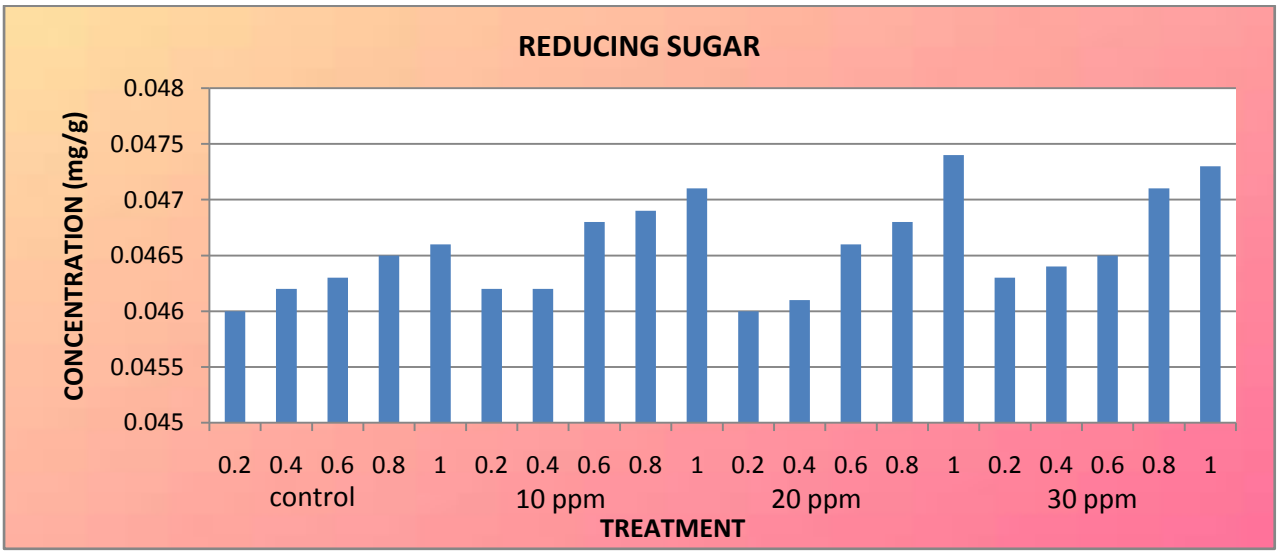
Fig. 9: Reducing sugar of the selected plant



This figure shows that the concentration of reducing suger with effect of salicylic acid. Concentration of reducing sugar was observed higher in all treated plants in all concentration as compared with control.

Table 10: Protein of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Protein (mg/g)
Control	0.2	0.1361
	0.4	0.1362
	0.6	0.1362
	0.8	0.1364
	1	0.1365
10 ppm	0.2	0.1362
	0.4	0.1362
	0.6	0.1363
	0.8	0.1363
	1	0.1363
20 ppm	0.2	0.1361
	0.4	0.1362
	0.6	0.1363
	0.8	0.1363
	1	0.1363
30 ppm	0.2	0.1361

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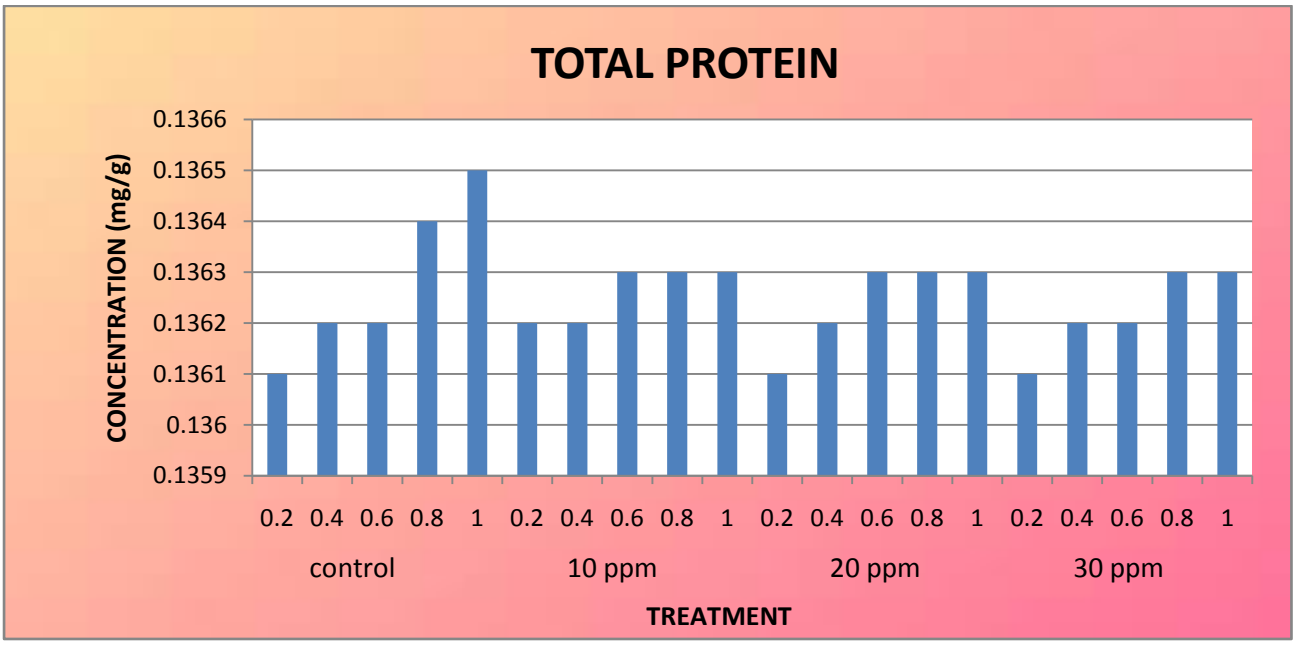
	0.4	0.1362
	0.6	0.1362
	0.8	0.1363
	1	0.1363

This table shows higher concentration of protein compared to the control.

Fig. 10: Protein of the selected plant



This figure shows that the concentration of protein with effect of salicylic acid. Concentration of total protein was higher in 0.6 (mg/g) concentrations in all treated palnts and lower in 1 (mg/g) concentration in treated plant as compared with control.

Table 11: Starch of the selected plant

Treatment Of Salicylic Acid /Concentration	Time Duration	Starch (mg/g)
Control	0.2	0.044
	0.4	0.0442
	0.6	0.0443
	0.8	0.0444
	1	0.0445
10 ppm	0.2	0.0443
	0.4	0.0443
	0.6	0.0444
	0.8	0.0445

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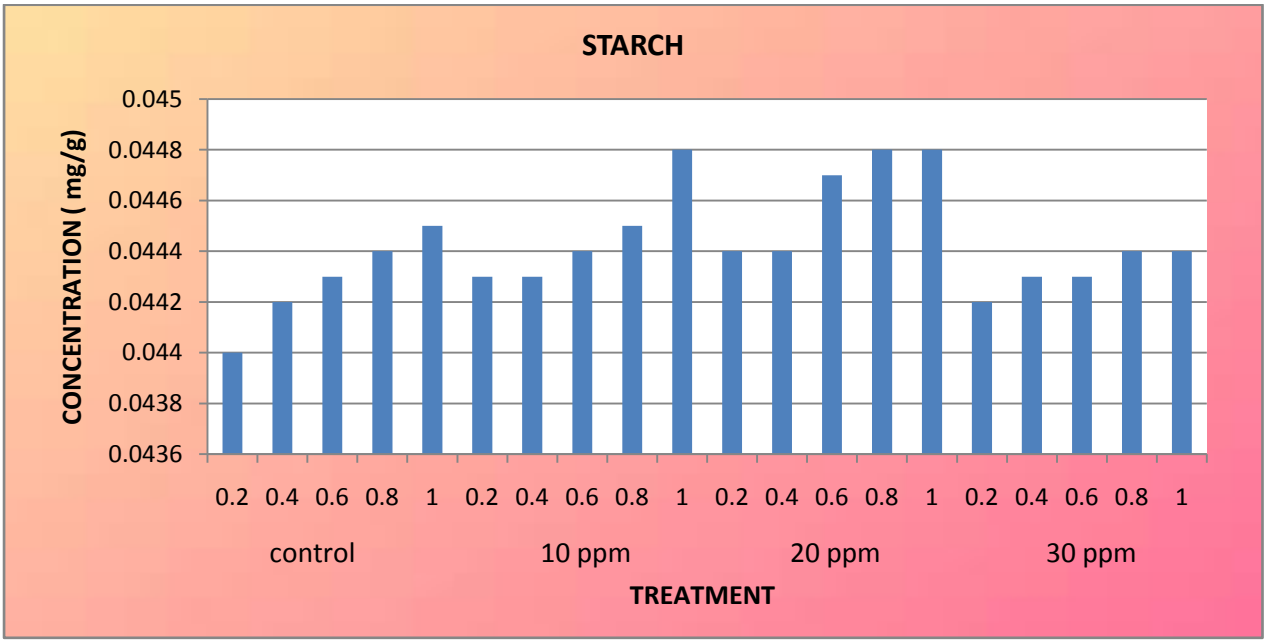
	1	0.0448
20 ppm	0.2	0.0444
	0.4	0.0444
	0.6	0.0447
	0.8	0.0448
	1	0.0448
30 ppm	0.2	0.0442
	0.4	0.0443
	0.6	0.0443
	0.8	0.0444
	1	0.0444

This table shows higher concentration of starch compared to the control

Fig. 11: Starch of the selected plant



This figure shows that the concentration of starch with effect of salicylic acid. Concentration of starch was higher in all treated plant in all concentration as compared with control plants.

Table 12: Chlorophyll of the selected plant

CONTANT	O.D. AT 663	O.D. AT 652	O.D. AT 645
CONTROL	0.589	0.213	0.183
10 ppm	0.232	0.175	0.222
20 ppm	0.618	0.166	0.225
30 ppm	0.246	0.162	0.235

This table shows higher concentration of chorophyll compared to the control

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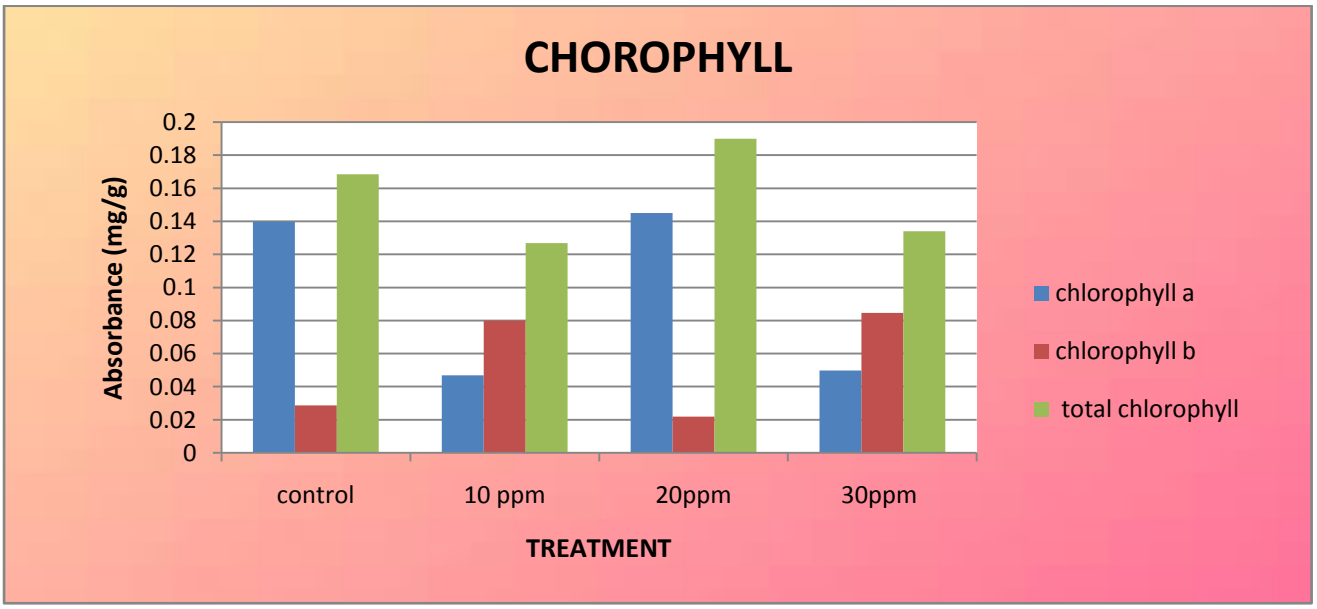
Fig. 12: Chlorophyll of the selected plant



This figure shows that the concentration of chorophyll a, chorophyll b, total chorophyll, with effect of salicylic acid. Concentration of chorophyll a was higher in 20 ppm, in Concentration of chorophyll b was higher in 30 ppm, Concentration of total chorophyll was higher in 20 ppm in treated plants as compared to control.

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