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Impact of Salicylic Acid and Its Application Methods on Some Morphological and Physiological Growth Properties of Two *Petunia* species

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Master's Degree of Science in Biology

By

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Certificate

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَسَلَكَهُ يَنَابِيعَ فِي الْأَرْضِ ثُمَّ
يُخْرِجُ بِهِ زَرْعاً مُّخْتَلِفاً أَلْوَانُهُ ثُمَّ يَهِيجُ فَتَرَاهُ مُصْفَرّاً ثُمَّ يَجْعَلُهُ
حُطَاماً إِنَّ فِي ذَلِكَ لَذِكْرَى لِأُولِي الْأَلْبَابِ ﴾

صَدَقَ اللَّهُ الْعَلِيِّ الْعَظِيمِ

سورة الزمر (٢١)

Dedication

To who he gives me the power to gain this knowledge and blessing me much more than I deserve , almighty "Allah"

To the person who remove the thorns from my path to pave the way of knowledge, the big heart (my dear father)

To the symbol of love and tenderness , the pure white heart (my beloved mother)

To the soul of hope , my soul mate (my dear sister)

To the person who gives me patience and strength (my dear brother)

To the pure and kind hearts , the roses of my life (my sister's and my brother's children)

To the city of peace (Baghdad)

ZAHRAA

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-Abstract-

The experiment was conducted in a greenhouse at Al Muthanna University, department of biology, College of Education for Pure Sciences during the grown season in December 2018. The aim was to study the effect of salicylic acid at (0,100, 200 and 300) ppm apply at (seed soaking, foliar spraying, seed soaking & foliar spraying) on growth some physiological and anatomical aspects of *Petunia* namely *P.hybredia* and *P.niglofera*. the project was carried out according to the completely random design (C.R.D) in three replicates. The results indicates the significant effect of salicylic acid at the lower concentration (100 ppm) when use to seed soaking & foliar spraying *P.hybredia* was more influence than *P.niglofera* on vegetative growth aspect like plant height (30.80 cm), root length (24.30 cm), fresh weight of shoot and root (27.40, 9.20 g) respectively, and the physiological aspect like chlorophyll a,b,a/b,a+b and carotene beside to the other aspect like relative water content (183.28)%, membrane stability (68.40 %), ion content of nitrogen (10.357mg/g) potassium (5.00 mg/g) sodium (3.833 mg/g) calcium (10.5mg/g) phosphate (1.060mg/g), carbohydrate (88.33 mg /g), protein (64.73mg/g), the permeability of ion like Na^+ , K^+ salicylic acid was show positive effects in reducing of permeability of K^+ and Na^+ ions (30.267, 4.617) while the result of anatomical side show that the salicylic acid increased the leaf area (15.333cm^2) and the number of stomata (55200). The least significant effect of the salicylic acid at concentrations (300 ppm) for seed soaking treatment share in *P.niglofera* for the growth axial like plant height (13.60 cm), root length (15.867cm), fresh weight of shoot (16.55g) and root (0.36 g), and the physiological aspect like chlorophyll a,b,a/b,a+b and carotene plus the other aspect like relative water content (46.15)%, membrane stability (25.50%), mineral content the nitrogen (1.16mg/g), potassium (1.66mg/g), sodium (1.050mg/g) calcium (3.1mg/g), phosphate (0.42mg/g) carbohydrate (44.00mg/g) protein (8.8mg/g) while the permeability of ion like (Na^+ , K^+) salicylic acid was show significant effects in reducing the K^+ and Na^+ (98.9, 39.667). The results of anatomical side shows that the salicylic acid increased the leaf area (5.00cm^2) and the numbers of stomata (18960).

List of Contents

No.	subject	Page
Chapter One :Introduction		
1.1	Introduction	1
1.2	The Aim of The Study was to Investigate	2
Chapter Two: Literatures Review		
2	Literatures Review	3
2.1	Solanaceae Plants	3
2.2	<i>Petunia</i>	4
2.2.1	<i>Petunia</i> Morphology	5
2.2.2	Classification of <i>Petunia</i>	5
2.2.3	Important of <i>Petunia</i>	6
2.2.4	Environment of <i>Petunia</i>	6
2.3	Salicylic Acid	7
2.3.1	Chemical Structure of Salicylic Acid	7
2.3.2	Biosynthesis of Salicylic Acid in Plants	8
2.3.3	Salicylic Acid as Plant Hormone (Phytohormone)	10
2.3.4	Effect of SA on the Vegetative Growth	11
2.3.5	Effect of SA on the Physiological Traits	12
2.3.5.1	Chlorophyll Content & Carotene Content	12
2.3.5.2.	Mineral Elements Content	14
2.3.5.3	Carbohydrates & Protein Content	14
2.3.5.4	Stomata	15
Chapter Three: Materials and Methods		
3	Materials and Methods	16
3.1	Laboratory Apparatuses and Equipment	16
3.2	Chemicals and Reagents	18
3.3	Preparation of Soil	18
3.4	Determination Field Capacity of Soil	18

3.5	Preparation of Salicylic Acid	19
3.6	Experimental Design and Treatments	19
3.7	Plant Cultivation	19
3.8	Growth , Anatomical and Physiological Parameter	20
3.8.1	Anatomical Characteristics	20
3.8.2	Leaf Area	21
3.8.3	Leaf Relative Water Contents	21
3.8.4	Estimation of Chlorophyll Type and Carotenoid Content	21
3.8.5	Measurement Percentage of Membrane Stability	22
3.8.6	Total Soluble Protein	23
3.8.7	Estimation of Carbohydrates	23
3.8.8	Digestion of Plant Sample to Determine Mineral	23
3.9	Statistical Analysis	24
3.10	Experimental Design	25
Chapter Four :Results		
4	Results	26
4.1	Effect of Salicylic Acid on Anatomical Properties	26
4.1.1	Epidermal Hairs Distribution on the Surface of leaves and Stem <i>Petunia</i> sp.	26
4.1.2	Stomata type in the <i>Petunia</i> sp.	28
4.1.3	Effect of SA on Leaf Area of <i>Petunia</i> sp	29
4.1.4	Effect of SA on Number of Stomata in <i>Petunia</i> sp	30
4.2	Effect of SA on the Growth Properties	32
4.2.1	Effect of SA on the Shoot Height of <i>Petunia</i> sp.	32
4.2.2	Effect of SA on the Root Length of <i>Petunia</i> sp.	34
4.2.3	Effect of SA on the Fresh Weight of Shoot	36
4.2.4	Effect of SA on the Fresh Weight of Root	37
4.3	Effect of SA on the <i>Petunia</i> Plant Physiological Properties	39

4.3.1	Effect of SA on Water Content of Leaf Tissue	39
4.3.2	Effect of SA on Stability of Plasma Membranes	41
4.3.3	Effect of SA on the Permeability of Potassium(k^+)	43
4.3.4	Effect of SA on the Permeability of Sodium Ions(Na^+)	44
4.3.5	Effect of SA on Chlorophyll a Content	46
4.3.6	Effect of SA on Chlorophyll b Content	48
4.3.7	Effect of SA on Chlorophyll a/b Content	50
4.3.8	Effect of SA on Chlorophyll a+b Content	52
4.3.9	Effect of SA on Carotene Content	54
4.3.10	Effect of SA on Carbohydrate Content	56
4.3.11	Effect of SA on Na^+ Content of <i>Petunia sp.</i> Plant	59
4.3.12	Effect of SA on Calcium Ca^{+2} Content of <i>Petunia sp.</i> Plant	61
4.3.13	Effect of SA on Nitrogen N^+ Content of <i>Petunia sp.</i> Plant	63
4.3.14	Effect of SA on Phosphate p^{-2} Content of <i>Petunia sp.</i> Plant	65
4.3.15	Effect of SA on Potassium k^+ Content of <i>Petunia sp.</i> Plant	67
4.3.16	Effect of salicylic acid on Protein Content	68
Chapter Five: Discussion		
5.1	Effect of SA on Anatomical Axial of <i>Petunia sp.</i>	71
5.2	Effect of SA on the Growth Axial of <i>Petunia sp.</i>	72
5.3	Effect of SA on the Physiological Axial of <i>Petunia sp.</i>	74
Chapter Six: Conclusions and Recommendations		
	Conclusions	78
	Recommendations	79
	References	80

List of Tables

No.	Subjects	Page
(3.1)	Apparatus Used in the Experiment and their Companies and Origin	19
(3.2)	Equipment Used in the Experimental and their Companies and Origin	20
(3.3)	Chemicals and their structure/ Companies and Origin	21
(4.1)	Effect of salicylic acid concentrations and treatments on the leaf area(cm^2) between the two species.	29
(4.2)	Effect of salicylic acid concentrations and treatments on the numbers of stomata between the two species.	31
(4.3)	Effect of salicylic acid concentrations and treatments on the height shoot (cm) in the <i>Petunia</i> sp.	33
(4.4)	Effect of salicylic acid concentrations and treatments on root length (cm) between the two species.	35
(4.5)	Effect of salicylic acid concentrations and treatments on fresh weight of shoot (g) between the two species.	37
(4.6)	Effect of salicylic acid concentrations and treatments on fresh weight of root (g) between the two species.	38
(4.7)	Effect of salicylic acid concentrations and treatments on water content (%) between the two species.	40
(4.8)	Effect of salicylic acid concentrations and treatments on stability of plasma membranes (%) between the two species.	42
(4.9)	Effect of salicylic acid concentrations and treatments on the permeability of potassium K^+ (%) from the leaf tissue between the two species.	43
(4.10)	Effect of salicylic acid concentrations and treatments on the permeability of Na^+ (%) from the leaf tissue between the two species.	45
(4.11)	Effect of salicylic acid concentrations and treatments on chlorophyll a (mg/g) between the two species.	47
(4.12)	Effect of salicylic acid concentrations and treatments on chlorophyll b (mg/g) between the two species.	48

(4.13)	Effect of salicylic acid concentrations and treatments on chlorophyll a/b between the two species.	51
(4.14)	Effect concentrations of salicylic acid and treatments on chlorophyll a+b (mg/g) between the two species.	53
(4.15)	Effect of salicylic acid concentrations and treatments on carotene content (mg/g) between the two species.	55
(4.16)	Effect of salicylic acid concentrations and treatments on carbohydrate content (mg / g) in leaf tissue between the two species.	57
(4.17)	Effect of salicylic acid concentrations and treatments on concentration of Na ⁺ (mg / g) between the two species.	59
(4.18)	Effect of salicylic acid concentrations and treatments on concentration of Ca ⁺⁺ (mg / g) between the two species.	62
(4.19)	Effect of salicylic acid concentrations and treatments on concentration of nitrogen (mg / g) between the two species.	63
(4.20)	Effect of salicylic acid concentrations and treatments on concentration of phosphate (mg / g) between the two species..	65
(4.21)	Effect of salicylic acid concentrations and treatments on concentration of K ⁺ (mg / g) between the two species.	67
(4.24)	Effect of salicylic acid concentrations and treatments on the proteins content in (mg / g) in the leaf Tissue between the two species.	69

List of Figures

Figure No.	subjects	Page
(2.1)	Chemical structure of salicylic acid	8
(2.2)	Pathway for salicylic acid biosynthesis in plants	9
(2.3)	Structure of stomata	15
(3.1)	<i>P.hybrida</i> plant	20
(3.2)	<i>P.niglofera</i> plant	20
(3.3)	Experimental design	25
(4.1)	Spread of epidermal hairs on the surface of the leaves petiole under magnification 10x	26
(4.2)	Two hairs; hair is containing two cells more than three cells 20x	27
(4.3)	Stream cytoplasmic from bottom to up in whole plant 40x	27
(4.4)	Epidermal cells in the blade were zigzagging cells under magnification 40x	28
(4.5)	Elongated (Rectangular)cell under magnification 40x	28
(4.6)	Structure of stomata apparatus under magnification 40x	28

List of Abbreviations

Abbreviations	Terms
SA	Salicylic acid
ABA	Absciscic acid
Con.	Concentration
Sp.	Species
ppm	Parts per million
chl.	Chlorophyll

Chapter One

Introduction

1. Introduction

1.1 Introduction

Human need the plants. All animals related to need the plant make it possible for us to a civilize. Before we had cities one day someone had the bright idea to plant the plants we like to eat. When humans did that, they were able to stay in one place full time. Then came the cities and a huge system of agriculture to support millions of people. On other hand human need plants in his medicine or treatment for long time ago against all present diseases. Moreover, ornamental plants play an important role in the lives of people and societies because of their important role in recreational and aesthetic areas in public parks, and main streets, which gives great beauty to the cities, and have an impact on the psychological and mood of humans, herbaceous flowering plants .One of these plants used in public parks and corridors to give joy and vitality and the transfer of the sense of nature for its aesthetic impact in the gardens, divided by the containment of flowers to paper grass grown for the beauty of the leaves and their ability to cut and shape and decoration or flowering grass. They are cultivated for the beauty of their flowers such as *Petunia hybrida* and other ornamental plants (Robbins and Colt,2008).

Petunia popular bedding plants are herbaceous perennials, usually grown as annuals and belong to the family Solanaceae. *Petunia* has become one of the most popular garden plants. Its origin is considered to be in South America, most of the varieties grown today are hybrids (*Petunia hybrida*). Its origin was thought to be by hybridization between *P. axillaris* (the large white or night scented) and *P. integrifolia* (violet flowered) (Gautam, 2012).In the other hand , *Petunia* has played a central role in transformation research since the earliest reports of plant transformation (Corner, *et al.*, 2009).

In recent years salicylic acid has been the focus of intensive research due to its function as an endogenous signal mediating local and systemic plant defense responses against pathogens. It has also been found that salicylic acid plays a role during the plant response to abiotic stresses such as drought, chilling, heavy metal toxicity, heat, and

osmotic stress. In this sense, salicylic acid appears to be, just like in mammals, an ‘effective therapeutic agent’ for plants. Besides this function during biotic and abiotic stress, salicylic acid plays a crucial role in the regulation of physiological and biochemical processes during the entire lifespan of the plant. The discovery of its targets and the understanding of its molecular modes of action in physiological processes could help in the dissection of the complex salicylic acid signaling network, confirming its important role in both plant health and disease.(Rivas-San Vicente and Plasencia, 2011; Zhang and Li, 2019).

Salicylic acid acts as a potential non-enzymatic antioxidant as much as plant growth regulator, playing an important role in regulating several plant physiological processes. salicylic acid has been identified as a signaling component in numerous plant responses to stress, salicylic acid is also involved in the activation of the stress induced antioxidant system ,stimulates flowering in many plants, increase flower life, control ion uptake by roots and stomatal conductivity (Muthulakshmi and Lingakumar, 2017).

1.2The Aim of The Study was to Investigate:

1. The effectiveness of the chemical compound (salicylic acid) and which method is best to apply it to the plants (seeds soaking alone, spraying the plant alone or seeds soaking & plants spraying) , in addition test a group of salicylic acid concentrations, and which concentrations show significant impact low or high concentrations.
2. Spot light was focused on *Petunia sp.* as a model to use in the scientific researches, due to some criteria as a short life cycle, and is a member of the big plant family (Solanaceae) . Then study the leaf trait , type of stomata for this plant.

Chapter Two

Literature Review

2. Literatures Review

2.1 Solanaceae Plant

A group of plants under a particular family is put together to share these plants with a variety of features. So, some of these features are classified as cellular structures, stored materials and many similarities among them. One of the plant families is Solanaceae plants.

The name Solanaceae is derived from the genus *Solanum*, "the nightshade plant". The etymology of the Latin word is unclear. The name may come from a perceived resemblance of certain solanaceous flowers to the sun and its rays. At least one species of *Solanum* is known as the "sunberry". Alternatively, the name could originate from the Latin verb *solari*, meaning "to soothe" (Heywood, *et al.*, 2007, Yadav, 2016) the nightshade family, in the order of Solanales, is a family of dicotyledonous flowering plants, consisting of about 98 genera with approximately 2700 species, widely distributed in temperate and tropical regions. Plants are usually herbs to vines, lianas, epiphytes, shrubs, and trees. Stems are erect, procumbent or climbing, sometimes prickly, and rarely thorny (Shah, *et al.*, 2013) . Leaves are simple, divided, or compound, usually alternate. Blades are entire. Stipules are absent. Flowers are bisexual, actinomorphic, solitary, fascicled, or arranged to various cymes, rarely racemose, terminally, laterally, axillary, or alternately with leaves. Calyx is usually 5-lobed, persistent, and inflated or not after anthesis. Corolla is rotate, campanulate or funnelform, and usually 5-lobed. Stamens are isomerous with corolla lobes and alternate and usually inserted on corolla tube. Anthers are free or adnate, longitudinally dehiscent or pore dehiscent apically. Ovary is superior and 2-loculed, rarely 1-loculed or incompletely 3–5-loculed. Carpels are two and oblique. Ovules are numerous and in axile placenta. Styles are linear. Stigma is capitate, undivided, or 2-lobed(Shaheen, *et al.*, 2011; Martin, *et al.*, 2016) . Fruit is a berry or capsule and lid dehiscent or valvularly dehiscent. Seeds are numerous, disk-like or reniform, and flat. Embryo is erect or curved annularly. Endosperm is copious and fleshy (Xu and Chang, 2017).

Therapeutically, these are the most powerful known anticholinergics in existence, meaning they inhibit the neurological signals transmitted by the endogenous neurotransmitter, acetylcholine. Medicinally important species of the family Solanaceae belong to following genera namely: Solanum, Atropa, Capsicum, Datura, Withania, Hyoscyamus, Nicotiana and Miscellaneous. These species are extensively used for medicinal purpose throughout the country (Girardi Bauermann, 2016). The Solanaceae family is characteristically ethnobotanical, that is, extensively used by humans. It is important source of food, spice and medicine. However, solanaceae species are rich in alkaloids whose toxicity to humans and animals ranges from mildly irritating to fatal in small quantities (Shah *et al.*, 2013). In addition the family solanaceae contributes several important food, drug and ornamental plants to human race, hence this family is considered as economically important (Xu and Chang, 2017).

2.2 *Petunia*

The genus *Petunia* (Solanaceae) is a popular ornamental plant cultivated worldwide, but the taxonomy of the genus has not been clear throughout the years and has been changed a few times since the first description by Jussieu (1803). The name of *Petunia* is derived from ‘‘petum’’ or ‘‘betum’’, an original name for the tobacco plant, *Nicotiana tabacum*, that is closely similar to *Petunia nyctaginiflora* (now *P. axillaris*), that one of the two *Petunia* species firstly described (Stehmann *et al.*, 2009; Park, *et al.*, 2016) *Petunias* are often produced in northern climates during the late winter and early spring months, when light levels are low and ambient photoperiods are short, necessitating the use of supplemental lighting to promote flowering. Breeding efforts have been successful. (Warner, 2010) the genus *P. axillaris* and *P. integrifolia* are short-lived perennials that behave mostly as ephemeral annuals under natural conditions. Both species occur in similar habitats ranging from sandy soil and disturbed sites to crevices in rocks (Dell’Olivo *et al.*, 2011) color is perhaps the most obvious difference between flowers of *P. integrifolia* and *P. axillaris*, and it is also genetically and biochemically the best characterized trait in *Petunia*. According to Wijsman’s classical studies (Wijsman 1983) *Petunia* (*Petunia hybrida*) is an important ornamental plant and an excellent model for investigating floral pigmentation which ranges wide from pink to red to purple, due mainly to anthocyanins (Ma, *et al.*, 2017; Kishimoto, *et al.*, 2019).

2.2.1 *Petunia* Morphology

Petunias are tender perennials. They form self-branching mounds that are bushy to cascading(1–2 ft) high. Their leaves are ovate to ovate-lanceolate(4–5 in.) long. The trumpet-shaped flowers may be single or double (2-5 in.) across. They come in all shades and may be edged, striped, or starred with a contrasting color. Some are sweet smelling. Even though petunias are perennials they are usually treated as annuals and raised from seed yearly (Kole, 2011).Flowers solitary in axils of leaves or leaf-like bracts on pedicel (3–5 cm) longer than the subtending leaves; calyx deeply 5-parted, 1–1.8 cm by 3.5 mm; corolla funnel form with 5 rounded, spreading lobes 5–7 cm, white, red, yellow, pink, purple, or variegated (Plates 1 , 2 , 3 , 4 , 5 and 6) sometimes fragrant; stamens 5–1 short (staminode), 2 medium and 2 long; style slightly exceeding stamens. Capsules conical, 2-valved, 8–12 mm long, dehiscent. Seeds many subglobose, 0.5 mm across (Lim, 2014).

2.2.2 Classification of *Petunia*

The classification of *Petunia sp.* as follows: (Kadereit and Bittrich, 2016)

Kingdom : Plantae

Superdivision : Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass : Asteridae

Order : Solanales

Family : Solanaceae

Genus: *Petunia* Juss.

Species: *Petunia hiberida*

Petunia niglofera

2.2.3 Importance of *Petunia*

Petunia is considered to be the first cultivated bedding plant and remained as a commercially important ornamental since the early days of horticulture and it is one of the favorite genera for developing new varieties. The history of petunia development as a crop is highlighted with implications for a wide variety of other floricultural crops (Warner, 2010). The petunia offers the potential for high economic returns and is an excellent source of additional income to the flower industry (Zhang *et al.*, 2012). The nutritional properties of *Petunia* (*Petunia hybrida* L.) are not well known and further uses of *petunia* need precautions (Chrysargyris *et al.*, 2019).

2.2.4 Environment of *Petunia*

South America is origin where the various species have been documented from emollitions are made in Argentina, Brazil, Bolivia, Paraguay, and Uruguay. The majority of the species have been found in three Brazilian provinces: Parana, Santa Catarina, and Rio Grande do Sul, and in areas of the other countries, particularly in interior areas where they share a common border (Frankel, *et al.*, 1988; Drummond, *et al.*, 2015). *Petunias* are good to outstanding annual flowers in gardens used as annual bedding and container plants in temperate zones (Sardoei *et al.*, 2014).

The lifecycle of petunia from seed to seed of only 3– 3,5 months can easily be obtained, allowing the growth of up to four generations a year. It has been shown for petunia that the rate in progress to flowering increased linearly with increasing temperatures. In practice, they found that lower temperatures have a double impact on generation time, since they do not only delay flowering, but also strongly delay ripening of the seedpods. To reach such a short generation time, they culture *Petunia* at relatively high temperatures (Vandenbussche *et al.*, 2016).

2.3. Salicylic Acid

Salicylic Acid is a phenolic derivative, distributed in a wide range of plant species. It is a natural product of phenylpropanoid metabolism. Decarboxylation of transcinnamic acid to benzoic acid and its subsequent 2- hydroxylation results to SA. It undergoes metabolism by conjugating with glucose to SA glucoside and an ester (Hayat *et al.*, 2007).

The first commercial production of synthetic SA began in Germany in 1874 (Asghari and Aghdam, 2010). Aspirin, a close analog of salicylic acid, was introduced by the Bayer Company in 1898 and rapidly became one of the most popular pharmaceutical preparations in the world. During the 19th century many compounds belonging to the group of salicylates were isolated from a variety of plants. Aspirin, a trade name for acetylsalicylic acid (ASA), undergoes spontaneous hydrolysis to SA. Exogenously applied it is rapidly converted to SA. Despite the fact that aspirin was not identified as a natural product, it is widely used by many plant scientists in their experiments. The reason is the similarity in their physiological effects (Elmagbri, 2016).

2.3.1 Chemical Structure of Salicylic Acid.

Phenolic are usually defined as substances that possess an aromatic ring bearing a hydroxyl group or its functional derivate , SA is a crystalline powder that melts at 157–159 C°. It is moderately soluble in water and very soluble in polar organic solvents (Ekinci, *et al.*, 2011).

Salicylic acid or ortho-hydroxybenzoic acid and related compounds belong to a diverse group of plant phenolic , Salicylates from plant sources have been used in medicines since antiquity. In 1828, in Munich was isolated for the first time a small amount of salicin (Raskin, 2003). Salicin (C₁₃H₁₈O₇) is an alcoholic β-glycoside that contains D-glucose with anti-inflammatory activities that is produced from all willow bark (Ashraf *et al.*, 2010), the glucoside of salicyl alcohol, from willow bar. Ten years latter Raffaele Piria named it SA, from the Latin word Salix for willow tree. The first

commercial production of synthetic SA began in Germany in 1874 (Popova, *et al.*, 1997).

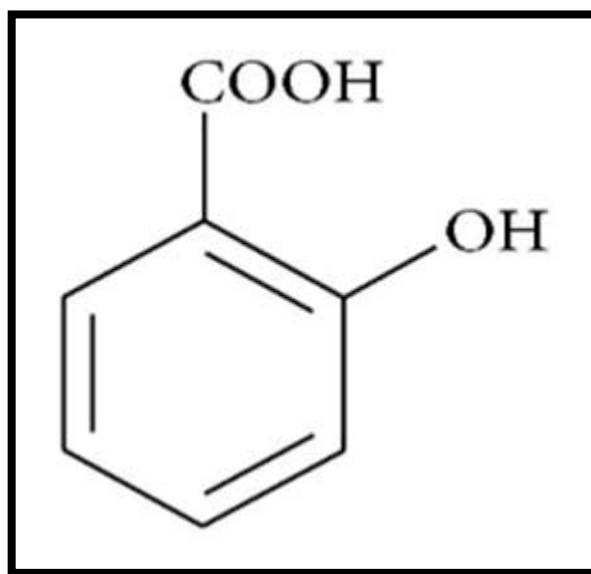


Figure (2.1) chemical structure of SA (Hayat, *et al.*, 2013).

2.3.2 Biosynthesis of Salicylic Acid in Plants

Salicylic acid is a key element in the complete metabolic processes in plant cells, so the plant seeks to build it naturally. It is largely believed that SA (ortho-hydrobenzoic acid) is a natural derivative of cinnamic acid, an intermediate in shikmic acid pathway, operative for the synthesis of phenolic compound (Zhang and Li; 2019).

- (i) Decarboxylation of the side chain of cinnamic acid to generate benzoic acid, that undergoes hydroxylation, at C-2 position. Recently, this scheme, for the synthesis of SA has been reported in tobacco plants (Yalpani *et al.*, 1992).
- (ii) Hydroxylation of cinnamic acid to o-coumaric acid followed by its decarboxylation to salicylic acid. The conversion of cinnamic acid to o-coumaric acid is believed to be catalyzed by trans-cinnamate-4-hydroxylase (Alibert and Ranjeva, 1971; and Alibert *et al.*, 1972) that was first detected in pea seedlings (Russel and Conn, 1967). labeled SA in *Gaultheria procumbens* (Ellis and Amrchein, 1971).

This observation strongly favours the belief that SA is synthesized from cinnamic acid, mediated by benzoic acid as an intermediate but El-Basyuni *et al.*, (1964) believe that both the above systems are operative, in higher plants, in the synthesis of SA ever, two possible routes have been proposed in this direction (Chen, *et al.*, 2009).

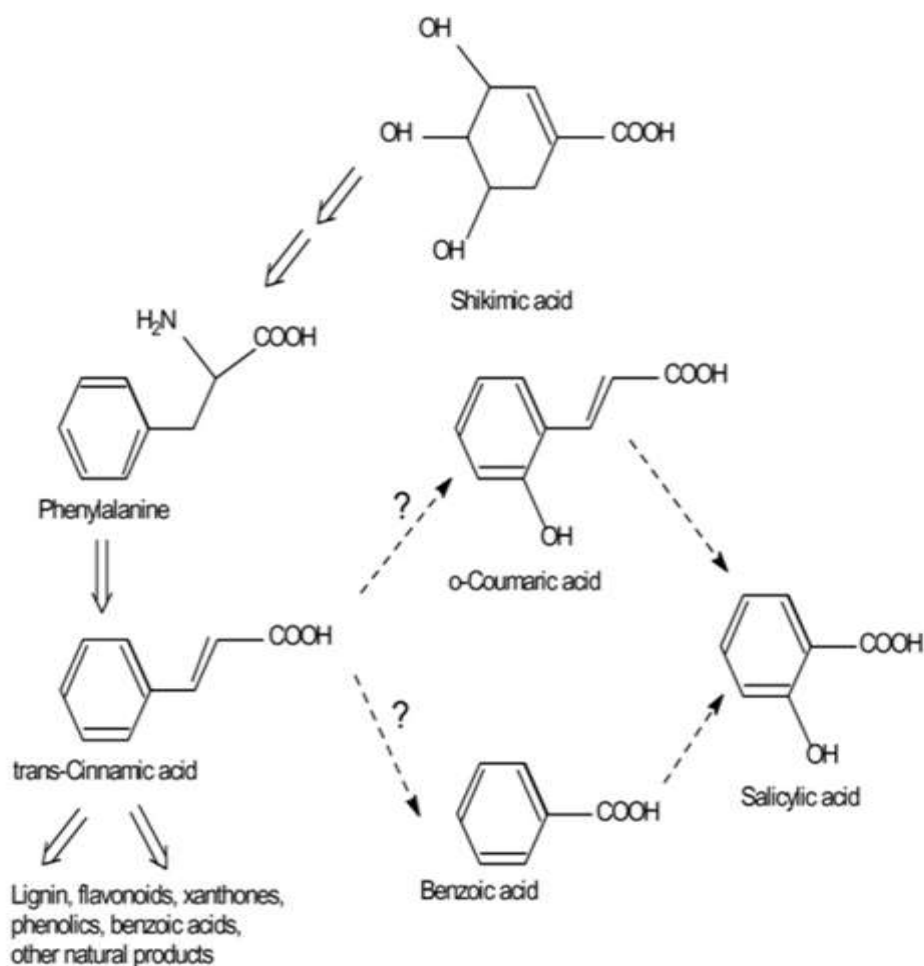


Figure (2.2): Pathway for salicylic acid biosynthesis in plants(Hayat *et al.*, 2007).

2.3.3 Salicylic Acid as Plant Hormone (Phytohormone)

Phytohormones are very much involved in directing the plant growth, in a coordinated fashion in association with metabolism that provides energy and the building blocks to develop the form that they are recognize as plants. Out of the recognized hormones, attention has largely been focused on auxins, gibberellins, cytokinins, abscisic acid, ethylene and more recently to brassinosteroids. However, a natural chemical- salicylic acid could be raised to the status of the above phytohormones because it has a significant impact on various aspects of the plant life. Salicylic acid was first discovered as a major component in the extracts from *Salix* (willow) ,whose bark from ancient time, was used as an anti-inflammatory drug. This acid (SA) is a phenol, ubiquitous in plants generating a significant impact on plant growth and development, photosynthesis, transpiration, ion uptake and transport and also induces specific changes in leaf anatomy and chloroplast structure. The SA is recognized as an endogenous signal, mediating in plant defense, against pathogens (Hayat *et al.*, 2008). The effect of exogenous SA depends on numerous factors such as the species and developmental stage of the plant, the mode of application, and the concentration of SA and its endogenous level in the given plant. Recent results show that not only does exogenous SA application moderate stress effects, but abiotic stress factors may also alter the endogenous SA levels in the plant cells. Salicylic acid is a potent signalling molecule in plants and is involved in eliciting specific responses to biotic and abiotic stresses (Kranterev *et al.*, 2006). SA has received a particular attention because it is a key signal molecule for expression of multiple modes of plant stress resistance. Although the focus has been mainly on the roles of SA on biotic stresses, several studies also support major roles of salicylates in modulation of the plant response to several abiotic stresses, such as UV light, drought, salinity, chilling stress and heat shock(Asghari and Aghdam, 2010).

2.3.4 Effect of SA on the Vegetative Growth

Salicylic acid has been shown to regulate various aspects of growth and development. Salicylic acid is one of the most important growth regulators that influence the detection and growth of roots and influence the absorption of nutrients and nutrients and has a role in increasing the root division of root populations and increasing the concentration of cytokines under stress conditions (Hayat and Ahmed, 2007). One of the reasons for increased plant height and stem diameter is the increase in concentrations of internal growth regulators such as auxins, gibberellins and cytokines and a decrease in the concentration of abscisic acid due to treatment with salicylic acid (Gharib and Hegazi, 2010). The application of salicylic acid (SA), acetylsalicylic acid (ASA) gentisic acid (GTA) or other analogues of SA, to the leaves of corn and soybean increase their leaf area and dry mass production but plant high and root length remained unaffected (Khan *et al.*, 2003). Fariduddin *et al.* (2003) observed the effect of a concentration of 10^{-5} M of salicylic acid contributes to an increase of salicylic acid contributes to an increase in dry matter accumulation in leaves of *Brassica juncea*, but above that it induces an inhibitory effect. Moreover, wheat seedlings, raised from the grains soaked in 10^{-5} M of SA possessed more number of leaves and higher fresh and dry mass, compared with water soaked, control (Hayat *et al.*, 2005). Likewise Afsana *et al.*, (2017) is found the morphological and yield contributing characters as well as yield of tomato were positively influenced with single and combined application salicylic acid (SA) and calcium (Ca^{2+}). Significant increase of plant height and number of leaves plant⁻¹ at 20, 40 and 60 DAT was observed with the application of A^3 treatment. Application of A^3 treatment also showed significant influence on production of cluster plant⁻¹ (20.44) flowers plant⁻¹ (168.1) and fruits plant⁻¹ (99.42) as well as fruit yield (72.57 t ha^{-1}). However application of A^4 treatment failed to improve the morphological and yield contributing characters as well as yield of tomato over the A^0 treatment (control). Shinwar *et al.*, (2018) observed Exogenously applied various levels of acetyl salicylic acid (ASA) including ASA^0 (Control), ASA^1 (0.30mM), ASA^2 (0.60mM), ASA^3 (0.90mM), ASA^4 (1.20mM) and ASA^5 (1.50mM) were investigated for induction of thermo-tolerance in tomato seedlings transplanted in different growth conditions, plant height (65.97 cm), stem diameter (1.69 cm), number of leaves plant⁻¹ (35.37), number of

flowers plant⁻¹ (88.61), number of fruits plant⁻¹ (39.05), total yield (28.48 tons ha⁻¹), and leaf relative water content (74.25%) was recorded in the open field. While investigating acetyl salicylic acid levels ASA⁴ (1.20 mM) treatment showed significant results and minimized the heat stress with maximum number of leaves recorded (36.28), flowers (105.53), total yield (30.97 tons ha⁻¹), chlorophyll content (62.24 µg.g⁻¹), and leaf relative water content (74%) as compared to heat stress (control). Predominantly, ASA⁴ (1.20 mM) produced better results with respect to growth and yield in both growth conditions by enhancing heat tolerance in tomato seedlings.

2.3.5 Effect of SA on the Physiological Trails

Salicylic acid endogenous and exogenous lead to improve the physiological characteristics and this depends on the concentration of SA and its application to the plant as well as plant species

2.3.5.1 Chlorophyll Content & Carotene Content.

Fariduddin *et al.*, (2003) reported that lower concentrations of SA were found to be beneficial in enhancing the photosynthesis, growth and various other physiological and biochemical characteristics of plants. On the other hand, at higher concentrations, SA itself may cause a high level of stress in plants. For that reason, it suggests that SA alters various physiological functions and biochemical processes in plants for regulating their growth and productivity in relation to change of plant environment. Moreover the plants that were pre-treated with SA exhibited relatively higher values physiological parameters (Chlorophyll & Carotene) were increased in the treatment groups compared to the control group (Kaya and Yigit; 2014). The obtained data revealed that, all treatments caused significant increases in growth parameters and photosynthetic pigments. The maximum increases in total pigments are 26.12% and 11.96% for Sakha 53 and Giza 102 cultivars at treatments 50 and 100 mg/l salicylic acid respectively that is found by (Mona, *et al.*, 2012). Shinwari *et al.*, (2018) found Exogenously applied various levels of acetyl salicylic acid (ASA) including ASA⁰ (Control), ASA¹ (0.30mM), ASA² (0.60mM), ASA³ (0.90mM), ASA⁴ (1.20mM), ASA⁵ (1.50mM) were investigated for induction of thermo-tolerance in tomato seedlings, indicated that chlorophyll contents was affected significantly by both ASA treatments and growth

conditions. However, their interaction is recorded non-significant at ($p \leq 0.05$) level of significance. Mean values for chlorophyll contents of ASA treatments stated that the highest chlorophyll content ($62.24 \mu\text{g.g}^{-1}$) is recorded in ASA^4 (1.20mM) while lowest chlorophyll content ($42.66 \mu\text{g.g}^{-1}$) is observed in ASA^0 (control). Among the mean values of growth conditions maximum chlorophyll contents ($58.78 \mu\text{g.g}^{-1}$) are found in open field while minimum chlorophyll contents ($45.63 \mu\text{g.g}^{-1}$) were observed in plastic tunnel. The application of SA (20 mg ml^{-1}) to the foliage of the plants of *Brassica napus* improved the chlorophyll contents (Ghai *et al.*, 2002). Similarly, soaking the grains of wheat in 10^{-5}M of SA resulted in the plants with higher pigment contents which declines as the concentration of SA is increased above that (Hayat *et al.*, 2005). Moreover, 30 days old plants of *Brassica juncea* sprayed with 10^{-5}M of SA possessed chlorophyll 20% higher than those sprayed by water only. However the maximum concentration (10^{-3}M) decreased the chlorophyll contents and the values were below that of water sprayed control, at 60 day stage (Fariduddin *et al.*, 2003). However, the leaves of corn and soybean applied with acetyl salicylic acid (ASA) or gentisic acid (GTA), exhibited no change in their chlorophyll contents (Khan *et al.*, 2003).

Zarghami *et al.*, (2014) noted the use of SA on *Petunia* obtaining the highest and lowest flower number and diameter produced by Sonja pink which sprayed with 2mM salicylic acid and Iranian variety with zero mM salicylic acid. The highest corolla tube, chlorophyll content, plant height, leaf area, root and shoot dry weight and biomass, stomatal conductivity, primary and secondary shoots produced by Iranian variety and 2mM salicylic acid while the lowest belonged to Sonja pink and zero level of salicylic acid. The highest and lowest electrolyte leakage observed for Sonja pink with zero level of salicylic acid and Iranian variety with 2mM salicylic acid. Salicylic acid activated the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation but decreased the level of chlorophyll pigments, both in wheat and moong plant and also the ratio of chlorophyll a/b, in wheat seedlings (Moharekar *et al.*, 2003).

2.3.5.2 Mineral Elements Content.

Plant stress can be decreased by application of salicylic acid, an hormone involved in plant signaling. That is observed by Wani *et al.*, (2016) once of the weapon defense in the plant against biotic stress is accumulation of nutrients mineral as K^+ , Ca^{+2} , P, N and Mg^{+2} . Exogenous application of SA improved plant behavior in the presence of NaCl. Nevertheless, the best results in terms of growth, photosynthetic pigment concentrations and mineral nutrition (limitation of Na^+ accumulation and maintenance of K^+ and Ca^{2+} content) were obtained in response to the combined SA + Ca treatment. Although the involved physiological parameters varied depending on the considered cultivar, the results suggest that Ca^{2+} and SA may interact to reduce the stress experienced by the plant in the presence of NaCl. However, the inhibition of the absorption of potassium by oat roots, under the impact of SA, was dependent on the pH and the concentration of the element in the medium. This inhibition was more prominent at lower pH, suggesting higher activity of protonated form of salicylic acid (Harper and Balke, 1981; Gordon *et al.*, 2002).

2.3.5.3 Carbohydrates & Protein Content

Salicylic acid plays an important role in the adjustment of apoplastic proteins and antioxidant enzymes associated with resistance to cold stress (Tasgin *et al.*, 2003). Reduction or increase in the production of protein decomposition can be attributed to the increase or decrease in antioxidant activity (El-Tayeb, 2005). However, the accumulated protein as a result of salicylic acid application in the rye had no role in antifreeze activity (Yu, *et al.*, 2001), and role of salicylic acid in resistance to frost is still unknown and further research is needed. The enzyme responsible for the synthesis of starch (starch synthase) is activated by potassium. So, when the amount of potassium is inadequate, the level of starch reduces while soluble carbohydrate and nitrogen compounds accumulate. Different concentrations of salicylic acid and its effective period cause multiple reactions in plant (Hare and Cress, 2004; and Senaratna *et al.*, 2003). It has been reported that treatment with salicylic acid increased proline in walnut and wheat (Singh and Usha 2003). Although salicylic acid decreased proline content in okra; the

plants treated with salicylic acid produced higher proline content than untreated plants under drought stress conditions (Ashraf and Foolad (2007). Free radical production increases under stress conditions and causes a disruption to electron transport in chloroplasts and mitochondria, and causes destruction and collapse of the membranes under stress conditions (Davies, 1987; Hassibi *et al.*, 2007). Increase in proline content under stress conditions as a result of increase in proline synthesis or decrease in decomposition is to cope with salinity. In general, proline accumulation under salinity and drought conditions has been reported by many researchers. It has been reported that proline adjusted secretion under osmotic conditions (Meloni *et al.* 2001). Furthermore, it was reported that sodium chloride increased soluble sugars in barley and tomato (Gao *et al.*, 1998; Ahmadi, *et al.*, 2107).

2.3.5.4 Stomata

Stomata are playing crucial role in land plant productivity and survival. stomatal apparatus is consist from two guards cells and pore between them with subsidiary cells. The main function of stomata is to open and close the pores in the leaves for an exchange of gases. It allows the plant to take in carbon dioxide and give out oxygen for photosynthesis.

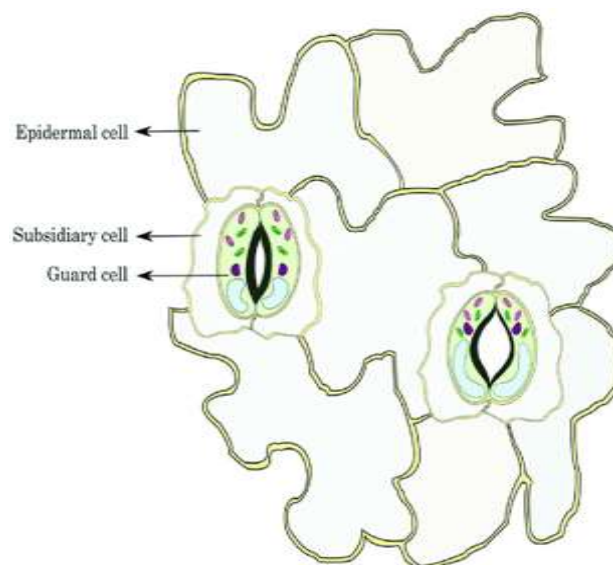


Figure (2.3) Structure of stomata .(Lima *et al.* 2018)

The opening and closing of stomata is regulated by the integration of environmental signals and endogenous hormonal stimuli. The various different factors to which the guard cells respond translates into the complexity of the network of signaling pathways that control stomatal movements (Daszkowska-Golec and Szarejko, 2013). The phytohormone SA is a phenolic compound that triggers systemic acquired resistance and confers drought tolerance to plants, the reason for closing stomata may be due to increased permeability of positive ions such as Ca^{+2} and k^{+1} , leading to shrinkage of the guard cells then closing the stomata (Miura *et al.*, 2013; Okuma *et al.*, 2014).

Chapter Three

Materials&Methods

3. Materials and Methods

3.1 laboratory Apparatuses and Equipment:

Below is a table showing Equipment and Apparatuses that used in this study .

Table(3.1): Apparatus Used in the Experiment and their Companies and Origin

sequence	Apparatuses	Company	Origin
1	Autoclave	Systec	Germany
2	Centrifuge	Universal	China
3	Analytical Balance	Kern	Germany
4	Distill water	LabTech	Korea
5	Flam photometer	LabTech	korea
6	Hood	LabTech	Korea
7	Hot plate magnetic stirrer	LabTech	Korea
8	Light microscop with camera	genex	china
9	micokjldahal	gerhardt	Germany
10	Water Bath	Lab Tech	Korea
11	Oven	Memmert	Germany
12	Electrical conductivity meter	Europe	Romania
13	Refrigerator	Concord	korea
14	Specterophotometer	Chrom tech	usa

Table (3.2): Equipment Used in the Experiment and their Companies and Origin

sequence	Equipment	Company	Origin
1	Slides	Sail brand	China
2	Cover Slides	Sail brand	China
3	Disposable surgical Gloves	Royal	Malaysia
4	funnel	Volac	England
5	Dust masks	Master guard	China
6	Motar and pestle	ISO lab	Germany
7	Filter paper	Zelpa	China
8	Volumetric flasks	ISO lab	Germany
9	Test tube	afco	Jordan
10	Timer	Junghans	Germany
11	Glass flasks and beakers	ISO lab	Germany
12	Parafilm	Rusco	U.K
13	Petri Dishes	QC lab	China
14	Graduated cylinder	Volac	England
15	Qauze wab	Hospital&homecara	China

3.2 Chemicals and Reagents

Table (3.3) shows the chemicals and reagents used in this study

sequence	Chemicals	Structure	Company/origion
1	Salicylic acid	$C_7H_6O_3$	Alpha/ india
2	Acetone	C_3H_6O	Chemlab/belgium
3	Phenol crystal	C_6H_6O	Isolab/ Germany
4	Sulfuric acid	H_2SO_4	Isolab/ Germany
5	Perchloric acid	$HClO_4$	Sdfcl/ india
6	glucose	$C_6H_{12}O_6$	Nice chemicals /india
7	Boric acid	H_3BO_3	Laboratory reagent
8	Ammonium molybdate	$(NH_4)_2MoO_4$	Laboratory reagent
9	Nitric acid	HNO_3	AAG chemical

3.3 Preparation of Soil

The soil was selected from the farms located by the banks of the Rumaitha river to conduct the study experiments. The surface soil is taken from a depth of 0-30 cm, dried out by an antenna and then passed through a sieve with diameters of 2 mm.

3.4 Determination field Capacity of Soil

The field capacity of the soil used in the experiments was estimated by taking a small, perforated, box and placed a filter paper at its base and weighting it, then placing 100 g of soil and then immersing the base of the container in a water container for a period of time. With water, and pulled out of the pot, and left until the last drop

of water pulled away, and then weighted again and the method of calculation as follows (Craze, 1990):

Soil water weight = Fresh soil weight - Dry soil weight

$$\text{Water content of soil (\%)} = \frac{\text{soil fresh weight} - \text{soil dry weight}}{\text{soil dry weight}} \times 100$$

3.5 Preparation of Salicylic Acid

SA is prepared by dissolving 1gm from SA in later distilled water to be concentration 1000 ppm, as stock solution. Along with this to reduce mistake in chemical preparations is using titration law ($N_1 \times V_1 = N_2 \times V_2$), to prepare the studied concentrations (0,100,200 and 300) ppm, also to distilled water stirrer then quantities were placed separately in a 1 liter flask of 1 liter for each treatment (Chemiasof, 2011).

3.6 Experimental Design and Treatments

The experiment was factorial based on a completely randomized design (CRD) with three replicates. There were nine treatments for each petunia cultivar plus the control one the treatment as follows (Khandan-mirkohi, Halbi and Salami, 2017), (Anaya *et al.*, 2018):

T₁=(100+200 and 300) ppm SA by seed soaking method.

T₂=(100+200 and 300) ppm SA foliar spraying method.

T₃=(100+200 and 300) ppm SA seed soaking + foliar spraying method

3.7 Plant Cultivation

This experiment was conducted under natural conditions in a Greenhouse of al Muthanna university /College of Education for Pure sciences by using two species of plant seeds *P. hybrida* and *P.niglefera*. Some of them are soaked in salicylic acid but other are not-soaking and are putt in pots that weight 4 kg in 3 replicates for each treatment.

- The plant cultivation is summers as following :
 1. Seeds were cultivated in 15/12/2018.
 2. Irrigation of the pots with 75% field capacity.
 3. Put more than 20 seed/pot.
 4. When the seedlings began to grow, reduce their number to 3 seedlings.
 5. Age for plant around 75 days in 1/3/2019 the plants were sprayed in SA.
 6. Spray each week for 6 weeks.
 7. The harvesting for plants in 1/5/2019 and then the practical experimental was conducted.



Figure (3.1) *P.hybrida* plant



Figure (3.2) *P.niglofera* plant

3.8 Growth , Anatomical and Physiological Parameter

3.8.1 Anatomical Characteristics

Samples were taken from the leaves of the plant and washed well by water. The upper epidermis and the lower epidermis were removed and placed on glass slides to create samples of the temporary slides, in order to identify the patterns of stomata in the leaf and distribute them on both sides of the leaf and the number of stomata (Cardoso, *et al.*, 2014).

3.8.2 Leaf Area

Cut fresh leave from the top of plant then weight it, after that cut a disc (1 cm²) from leave and weight ,leaf area is measured according to the following formula :

total leaf area=1cm² × weight of leaf / weight of the disc (Shaheen M.A,1987).

3.8.3 Leaf Relative Water Contents

The relative water content of the plant leaves was estimated by cut from the top of plant the third fully expand leave in the afternoon because the plant at this time had reached the true content of the water, the leaf weighed to determine its fresh weight, then leaves placing in distilled water for 12-16 h at room temperature for rehydration the excess water is then removed from the surface of the plant sample using filter paper and directly weighed to detect turgid weight .The leaf cells at the top of their turgid pressure at that time. Dry weight is determined after oven drying the leaf samples at 70oC for 24h.

RWC is calculated from(Schonfeld *et al.*, 1988) formula as follows:

$$RWC = (FW - DW) / (TW - DW) \times 100$$

FW = fresh weight of a leaf.

DW = dry weight of a leaf.

TW = turgid weight of leaf.

3.8.4 Estimation of Chlorophyll Type and Carotenoid Content

Chlorophyll and carotene contents are evaluated in fresh leaves by the method of (Arnon., 1949) depending on (Mackinney .,1941) a total of 200 mg of fresh tissue from inter venial leaf areas was crushed using a mortar and pestle containing 80% acetone. The optical density (OD) of the solution was recorded at 645 , 663nm for chlorophyll estimation and 452,5 nm for carotenoid estimation using a spectrophotometer (Chrom tech, USA).

The following relationships were used to calculate the amount of chlorophyll :

$$\text{Chl.a} = (12.7 (\text{D663}) - 2.69 (\text{D645})) \times V / (1000 \times W) .$$

$$\text{Chl.b} = (22.9 (\text{D645}) - 4.68 (\text{D663})) \times V / (1000 \times W) .$$

$$\text{Chl.(a+b)} = (\text{Chl.a}) + (\text{Chl.b})$$

$$\text{Carotenoids} = 4.2 \times \text{D452.5} - (0.0264 \text{ Chl.a} + 0.426 \text{ Chl.b})$$

D= the optical density reads of chlorophyll extracted at the wavelengths 663 and 645 nm, respectively

V= The final volume of the diluted acetone is (80%).

W= dry weight per gram of extracted vegetable tissue.

3.8.5 Measurement Percentage of Membrane Stability

The membrane stability determines by taking the uniform leaf discs cut out from randomly chosen plants per replicates were taken from the middle portion of the fully developed leaf and washed with distilled water to remove surface contamination then transfer this disc to a petri dish containing 5 ml of distilled water. Leave the petri at room temperature 10 °C, for 24 h then measure the conductivity in the solution by electrical conductivity meter ,the same samples are autoclaved at 121 °C for 20 min, and after cooling to 25 °C, the electrical conductivity of the left filter was re-measured and the proportion of the damage index is estimated by the equation of (Bandurska , 1998) as follows:

$$\text{MSI} (\%) = (T_1/T_2) / (C_1/C_2) \times 100$$

Where :C₁ and C₂ Represents the read of the electrical conduction of the control treatment before and after the tissue is killed.

T₁ and T₂ Represents the read of the electrical conduction of each treatment before and after the tissue was killed respectively.

The concentration of sodium and potassium ions was also estimated for leachates foliar tissue by flame Photometer.

3.8.6 Total Soluble Protein

The protein was estimated using the Follen method (Schacterale and Pollak, 1973) is modified from method (Lowry *et al.*, 1951) by measuring the visible density at 650 nm using the optical spectrometer.

3.8.7 Estimation of Carbohydrates

Weight of 0.20 g of well-grounded leave and mix with distilled water (10 ml) and then put in a centrifuge for 15 minutes / 3000 min⁻¹ cycle and aspirate the solution with filter paper, then pull 1 ml of leachate into a new test tube, add 5 ml of phenol at 5% and 5 ml of 80% sulfuric acid after stirring and shaking. The tubes are cooled and then photo resisted after proper dilution along a 488 nm wavelength using a spectro photometer (Al Wahbi and Bassallah, 2003).

3.8.8 Digestion of Plant Sample to Determine Mineral

Grind 0.5 g of the dry plant sample by the method (Alzoubi, *et al.*, 2013) and digest by wet digestion method by adding 5 cm³ of the sulfuric acid H₂SO₄ and HClO₄ as 1:4 and heat the sample using hot plate until the solution color is clear after filtration the sample prepared to estimate the mineral as following :

1. Measure the concentration of sodium, potassium and calcium in the plant

Sodium, potassium and calcium were measured in the plant using an optical flame photometer as described by (Richard, 1954).

2. Estimate the concentration of nitrogen

Nitrogen is estimated in the digested plant sample using Micro Kjeldahl as described by (Alzoubi, *et al.*, 2013).

3. Determine the concentration of phosphor

Phosphor determined in the vegetative extract of the plant using spectrophotometer as reported in (Richard, 1954).

3.9 Statistical Analysis

The experiments are designed and statistically analyzed using the Completely Randomized Design (CRD) experiment (Antar, 2010). The significant differences in transaction rates are compared using the Duncan's New Multiple Test under 0.05 significance level .

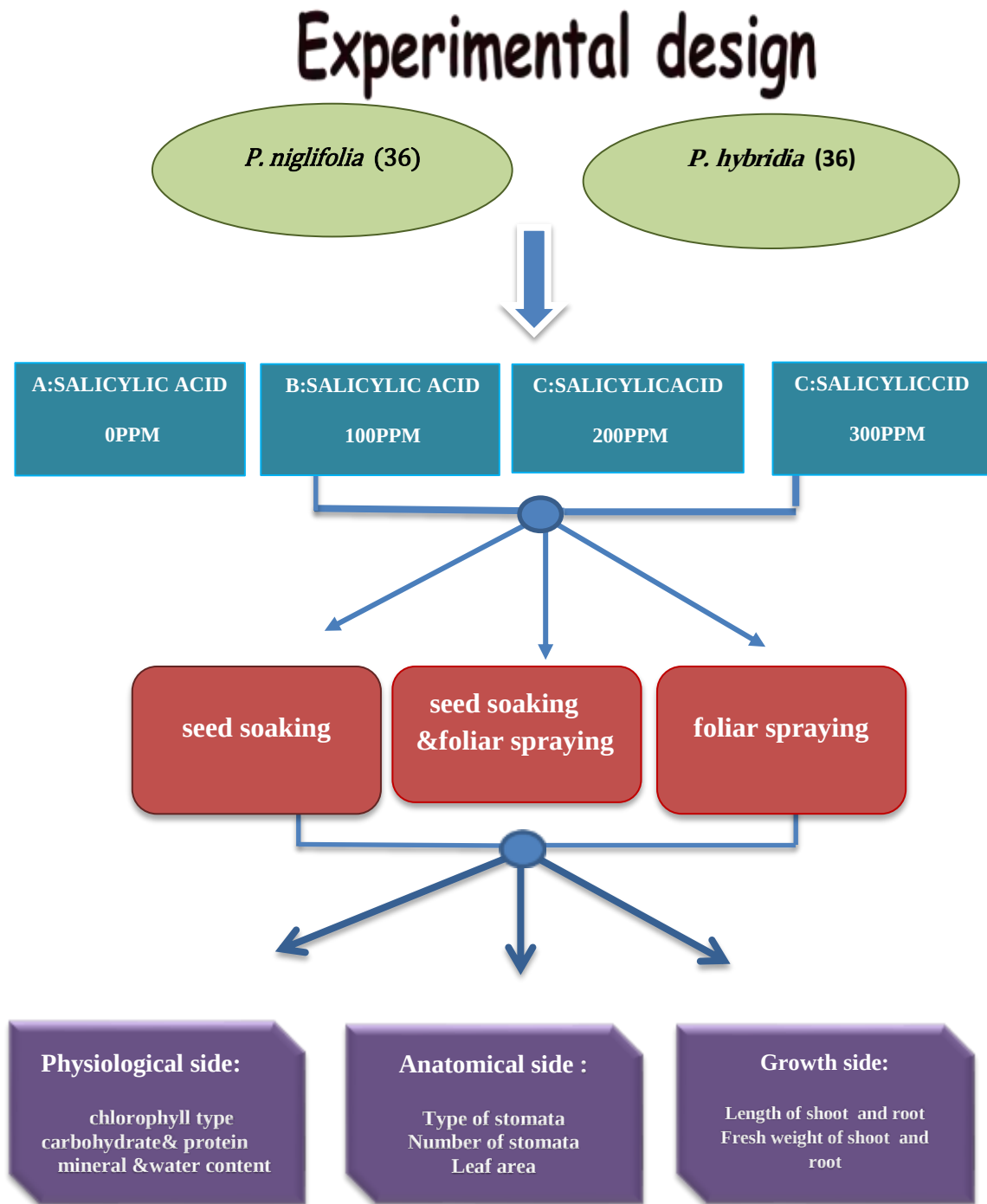


Figure (3.3): show experiment design

Chapter Four

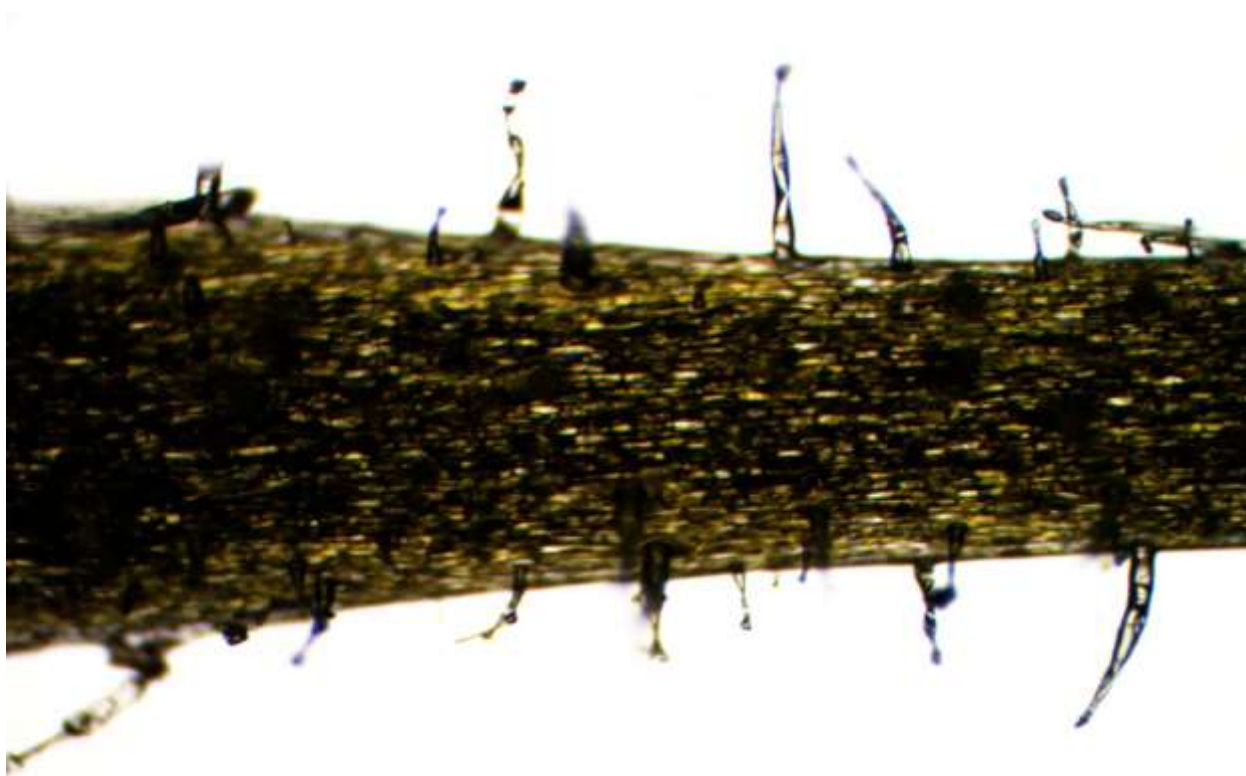
Results

4.Results

4.1 Effect of Salicylic Acid on Anatomical Properties

4.1.1 Epidermal Hairs Distribution on the Surfaces of Leaf and Stem of *Petunia* sp.

The epidermal hairs (Trichomes) that characterizes the plant leaf has a significant evolutionary and taxonomic value. The most common hairs are arising from an epidermal cells to form the hairs, which are spreading on the different organs of the body of the plant or limited to a member without the rest of the members as it may remain alive for a long time retention of its protoplasmic contents, or die and full of air, as figure (4.1) show.



Figure(4.1): spread of epidermal hairs on the surface of the leaves petiole under magnification 10x .

On the other hand figure (4.2) show the shape of hair is describing as simple, because it is without branching and it is almost consists from two or more than three cells, in addition it contains from three parts base, stalk and head, there is a glandular cell at the end of hair, therefore it is called glandular hair as figure (4.3) show.

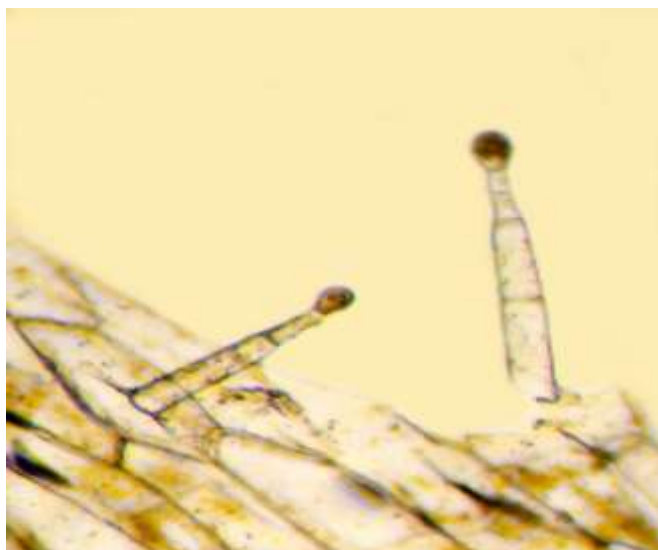
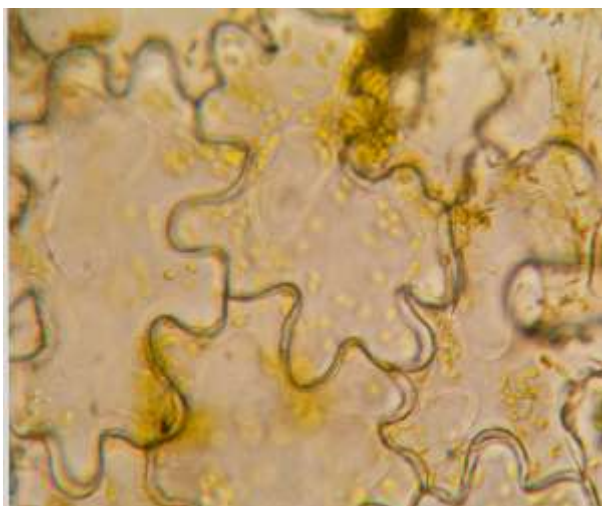


Figure (4.2): Two hairs; hair is containing two cells more than three cells 20x.



Figure (4.3): Stream cytoplasmic from bottom to up in whole plant 40x.

Ordinary epidermal cells: This type of cell represents the most common species in most plants, and it is considered to be less relevant than other species. On the other hand they are representing the ground which another types of epidermal cells. Actually all ordinary epidermal cells are differences in shape and size according to different of plants and even the once organ in the plant. The figures (4.4) and (4.5) shown difference between shape of cell in the petiole is usually like to be elongated (Rectangular) cells, but ordinary epidermal cells in the blade were zigzagging cells.



Figure(4.4) Epidermal cells in the blade were zigzagging cells under magnification 40x.



Figure (4.5) Elongated (Rectangular)cells under magnification 40x .

4.1.2 Stomata type in the *Petunia* sp.

Stomata are key innovations for the diversification of land plants. They consist of two differentiated epidermal cells or guard cells and a pore between that leads to an internal. Figure (4.6) show the type of stomata apparatus is Anisocytic type which means is containing three subsidiary cells surrounding guards cells, and its size are different from cell-1 small and second cell-2 is big then the third cell-3 is bigger than the other.

* All images of the anatomical side that was performed under the microscope related to the effect of salicylic acid at 100 ppm and (seed soaking +foliar spraying) method.



Figure (4.6): Structure of stomata apparatus under magnification 40x.

4.1.3 Effect of SA on Leaf Area of *Petunia* sp.

The results indicated in the table (4.1) The highest values were attributed to the plant species *P.hybrida* (15.333) cm², found in the third treatment T₃ (seed soaking + foliar spraying) of concentration 100 ppm and followed by the second treatment T₂ foliar spraying only of the same concentration so the value of leaf area here it was (12.967) cm². From the same table it clear that the other plant species *P.niglofera* also had the highest values of leaf area the value is (11.30) cm².

Table (4.1) Effect of salicylic acid concentrations and treatments on the leaf area(cm²) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	6.60 def	7.233 def	6.80 def	5.00 f	5.883 d		
	T ₂	6.60 def	8.60 de	7.30 def	5.267 f	7.25 c		
	T ₃	6.60 def	11.30 bc	8.933 cde	5.10 f	7.883 bc		
<i>P. hyberada</i>	T ₁	6.80 def	7.70 def	7.467 def	6.467 def	7.125 c		
	T ₂	6.80 def	12.967 b	8.733 de	6.367 ef	8.875 b		
	T ₃	6.80 def	15.333 a	9.233 cd	5.267 f	10.458 a		
Interference plant × Con.	<i>P. niglefera</i>	6.60 cd	8.988 b	7.711 bc	6.055 d		7.294 b	
	<i>P. hyberada</i>	7.333 cd	10.677 a	9.133 b	6.80 cd		8.53 a	
Interference treatment × Con.	T ₁	6.70 d	7.50 cd	7.083 d	6.833 d			
	T ₂	6.70 d	10.116 b	7.70 cd	6.366 d	7.504 b		
	T ₃	6.70 d	14.15 a	8.983 bc	6.116 d	9.666 a		
Effect of Con.		6.883 c	9.833 a	8.233 b	6.70 c			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test (P < 0.05).

Talking about the interaction between *Petunia* sp. and salicylic acid concentrations, the highest value of leaf area was obtain at 100 ppm (10.677) cm² and 200 ppm (9.133) cm² respectively for *P. hyberada* also the genus plan *P. niglefera* obtain the highest value of leaf area at 100 ppm (8.988) cm².

Certainly, the best values recorded in the interaction between the concentrations of salicylic acid and its treatment methods, will be found at the concentration of 100 ppm in treatment T₃ at (14.15) cm², and then the concentration of 200 ppm is

(8.983) cm^2 , for the same treatment, then the second treatment T_2 (foliar spraying) at concentration 100 ppm, note that from the data of table (5) there are significant differences at the level of probability of 5% because there are similarities in the letters, but they are numerically different, while the less valuable for leaf area in the SA concentration 300 ppm, and T_3 in the *P. nigrifera* it was (6.833) cm^2 .

Absolutely in case of concentrations effect, the concentration of 100 ppm is the best in terms of the positive effect and increase the leaf area and comes after 200 ppm, and so on for the rest of the other concentrations respectively.

Also, the interaction values between the plant and the three treatment methods, the results indicate that the T_3 treatment of the plant *P. hybrida* recorded the highest values by approximately (10.458) cm^2 , and gradually decreased the root weights of the plant to (7.125) cm^2 and indicate the lowest value at (5.883) cm^2 in the leaf area of the plant *P. nigrifera*, and there is significant differences between them at the probability level of 5%.

There are significant differences in the effect of plants *Petunia* sp. among them, the value at (8.53) cm^2 related to *P. hybrida* which is higher than the value (7.294) cm^2 related to *P. nigrifera*.

The effect of the treatments was evident from the significant differences between the results at the probability level of 5%. The third treatment T_3 recorded the highest effect on the leaf area of the plant approached (9.666) cm^2 compared to T_1 (seed soaking only) which is (6.566) cm^2 value.

4.1.4 Effect of SA on Numbers of Stomata in *Petunia* sp.

The results of the table (4.2) shows that the highest numbers of stomata for the plant leaf is (55200) stomata / leaf and (40680) stomata/ leaf in *P. hybrida* and *P. nigrifera* respectively in the same treatment seed soaking and foliar spraying method with 100 ppm of Salicylic acid. In additionally there are no significant differences between them at the level of probability of 5% because they took the same letters, but there is numerical differences. The lowest value of stomata for the plant leaf is (23210) stomata / leaf and 18960 stomata/ leaf in *P. hybrida* and *P. nigrifera* respectively in the same treatment seed soaking with 300 ppm of salicylic acid.

Table (4.2) Effect of salicylic acid concentrations and treatments on the numbers of stomata between the two species.

stomata between the two species.								
<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species× treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	23760 def	2628 0 def	26040 def	18960 f	21180 d		
	T ₂	23760 def	3096 0 de	27720 def	18960 f	26100 c		
	T ₃	23760 def	4068 0 bc	32160 cde	18000 f	28380 bc		
<i>P. hyberada</i>	T ₁	24480 def	3024 0 de	26880 def	23210 def	25650 c		
	T ₂	24480 def	4668 0 b	31440 de	23232 def	31950 b		
	T ₃	24480 def	5520 0 a	33240 cd	22920 ef	37650 a		
Interference plant × Con.	<i>P. niglefera</i>	23760 cd	3236 0 b	27760b c	21800 d		26260 b	
	<i>P. hyberada</i>	26400 cd	3844 0 a	32880 b	24480 cd		30710 a	
Interference treatment × Con.	T ₁	24120 d	2700 0 cd	25500 d	24600 d			23640 c
	T ₂	24120 d	3642 0 b	27720 bc	22920 d			27015 b
	T ₃	24120 d	5094 0 a	32340 bc	22020 d			34800 a
Effect of Con.		24780 c	3540 0 a	29640 b	24120 c			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Interaction between plant and concentrations of SA shows the highest number of stomata is in the plant variety *P. hyperida* in the concentration of 100 ppm (38440) stomata / leaf followed by a concentration of 200 ppm (32880) stomata / leaf, as well as the other variety the highest number of stomata in the concentration of 100 ppm is (32360) stomata / leaf. On the other hand there is no statistically significant differences due to the similarity of the letters next to the number.

From the same table the interference between treatments of salicylic acid and its concentrations the treatment T₃ is meaning (seed soaking + foliar spraying) at 100 ppm (50940) stomata / leaf there is significant differences at probability level 5% followed by values at (32340) stomata / leaf at, 200 ppm in the same treatment while the treatment T₂ foliar spraying take the value (36420) stomata / leaf which is also significant difference .

Explained the effect of four concentrations of SA, the concentration at 100 ppm have the highest values (35400) stomata /leaf compared to the control concentration,

followed by a concentration value (29640) stomata / leaf at 200 ppm, which is also significant difference.

So results of the interaction between *Petunia* sp. and methods treatment showed the highest value of interaction shows significant differences for the third treatment with a value (37650) stomata / leaf, followed by the second and the first are seen in the plant *P. hybrida*., while the highest value of the interaction is found in the third treatment, which is significantly different from the first show in *P.niglofera*

It turns out that effect of plants, the first class named *P.hyperida* have value (30710) stomata / leaf is superior to the second named *P.niglofera* at (26260) stomata / leaf.

Finally, The table confirms that the effect treatment there is significant differences between them at the probability level of 5% it was the highest value is (34800) stomata / leaf while the lowest value at (23640) stomata / leaf.

4.2 Effect of SA on The Growth Properties

4.2.1 Effect of SA on The Shoot Height of *Petunia* sp.

The results of the table (4.3) shows that SA resulted in a significant increase in the length of the plant, the highest value was the height of the *P. hyberada* plants, at 100 ppm, and in the method of seed soaking and spraying plants (T₃) it is (30.80)cm, while the second value is for the same method of treatment (T₃), but in the another concentration 200 ppm. On the other hand highest value of in the *P. niglefera* is (28.20) cm, in SA concentration 100 ppm in the third method treatment is meaning (seed soaking + foliar spraying) T₃.

Although there is no significant differences between values in terms of asymmetric letters associated with numbers they are numerically different, while it is less valuable for plant height in the SA concentration 300 ppm, and T₃ in the *P. niglefera* it is (13.60) cm. The values vary between different SA concentrations as well as different treatment methods, some of them are significant and others are not significant, although there is a significant difference.

Table (4.3) Effect of salicylic acid concentrations and treatments on the height shoot(cm) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference plants × treatment	Effect of species	Effect of treatment	
		0	100	200	300				
<i>P. niglefera</i>	T ₁	17.60 hi	18.10 hi	17.50 hi	13.60 j	17.458 d			
	T ₂	17.60 hi	24.967 cde	22.967 def	13.60 j	15.991 d			
	T ₃	17.60 hi	28.20 abc	25.633 bcd	13.60 j	22.375 b			
<i>P. hyberada</i>	T ₁	21.867 fgh	20.633 fgh	19.167 ghi	17.433 hi	19.975 c			
	T ₂	21.867 fgh	25.967 bcd	25.767 abc	16.633 ij	23.60 ab			
	T ₃	21.867 fgh	30.80 a	28.60 ab	16.30 ij	24.716 a			
Interference plant × Con.	<i>P. niglefera</i>	17.766 d	23.533 b	20.966 c	13.60 d		17.808 b		
	<i>P. hyberada</i>	17.766 d	26.722 a	26.40 a	18.90 d		23.563 a		
Interference treatment × Con.	T ₁	21.200 cd	20.233 cd	20.517 cd	15.60 e				19.183 c
	T ₂	21.200 cd	24.417 b	21.867 c	15.60 e				20.529 b
	T ₃	21.200 cd	28.383 a	24.883 b	18.733 d				22.345 a
Effect of Con.		20.650 b	23.844 a	22.65 a	15.6 c				

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Interaction between SA and plant species , the concentration of 100 ppm in the *P. hyberada* plants takes the highest values and differed significantly from the rest of the concentrations it is (26.722) cm, in another side lowest value is (13.60) cm in the *P. niglefera* with 300 ppm SA concentration and treatment method (seed soaking + foliar spraying).

The results of the interaction between the treatment methods and SA concentrations showed a significant superiority of the third treatment, namely seed soaking especially at 100 ppm compared with other treatments and concentrations, ranked second in terms of effect is the concentration of 200 ppm, if the values (28.383 and 24.883) cm respectively. The 100 ppm by foliar spraying only ranked third in terms of the high value figure that was (24.417) cm. The lowest values are for the concentration of 300 ppm which becomes (15.60) cm which it significantly different even from the control treatment it is (21.200) cm.

As for the effect of the four concentrations of SA, the concentrations of 100 ppm and 200 ppm are not significantly different, although they differed numerically as (23.844 and 22.65) respectively, while the lowest rise (15.6) at 300 ppm.

Concerning the interference between *Petunia* sp. and methods treatment a third treatment (seed soaking + foliar spraying) outperformed the rest of the treatments, especially the *P. hyberada* where the highest value reached (24.716) cm, while the lowest value was at the concentration of 300 ppm treatment of *P. niglefera* it is (17.458) cm.

While the preference was among the varieties of petunia plant for *P. hyberada* approximately (23.563) cm, but *P. niglefera* was (17.808) cm.

Finally, on the effect of the treatments, the third treatment (seed soaking+ foliar spraying) is significantly higher than the other treatments at 5% probability level. The top of the transactions with (22.345) cm value is superior to other transactions that are (20.529 and 19.183) cm values respectively.

4.2.2 Effect of SA on the Root Length

The data of table (4.4) shows that the root lengths of a plant has significant differences with different concentrations and treatments with salicylic acid. The highest value at 100 ppm approximately (24.30) cm followed by value (23.533) cm at 200 ppm for the same treatment (seed soaking + foliar spraying), both values belong to a plant (*P. hyberada*). When observing the table found there is significant differences and the values are less than the share of plant (*P. niglefera*), as it almost touched (12.00) cm.

Interference between plant varieties and salicylic acid concentrations, there are no significant differences between the highest values of both plants (20.155 and 19.355) cm, although there are numerical differences at the best concentration 100 ppm, also the lowest root length in a plant, which is at a concentration of 300pmm approximately (17.555) cm.

Table (4.4) Effect of salicylic acid concentrations and treatments on root length in (cm) for between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference plants × treatment	Effect of plants	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	12.00 j	18.20 efgh	18.067 efgh	15.867 hi	15.85 c		
	T ₂	12.00 j	19.00 defg	18.967 defg	15.867 hi	16.975 bc		
	T ₃	12.00 j	20.967 cd	20.467 cde	15.10 i	17.675 b		
<i>P. hyberada</i>	T ₁	15.60 hi	18.30 efgh	18.267 efgh	17.833 efgh	16.533 bc		
	T ₂	15.60 hi	21.567 bc	19.533 cdef	17.60 fghi	19.216 a		
	T ₃	15.60 hi	24.30 a	23.533 ab	16.633 ghi	19.641 a		
Interference plant × Con.	<i>P. niglefera</i>	15.60 c	19.355 a	18.777 ab	17.555 b		17.34 7 a	
	<i>P. hyberada</i>	12.00 d	20.155 a	19.866 a	17.877 b		17.95 0 a	
Interference treatment × Con.	T ₁	13.80 g	18.283 de	17.90 de	17.20 ef			
	T ₂	13.80 g	20.25 bc	18.983 cd	16.966 ef	17.325 b		
	T ₃	13.80 g	23.916 a	21.016 b	15.866 f	19.429 a		
Effect of Con.		13.80 d	20.011 a	19.066 b	17.716 c			

*Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Interaction between treatment and salicylic acid concentrations, values differ greatly from each other in terms of asymmetric letters associated with numbers. Likewise the highest value of interference is (23.916) cm, at a concentration of 100 ppm, and the lowest value is (13.80) cm for the control treatment.

Absolutely in case of concentrations effect, the concentration of 100 ppm is the best in terms of the positive effect and increase the length of the roots and comes after 200ppm and so on for the rest of the other concentrations respectively.

The results of the interaction between plants and methods of treatment with salicylic acid, shows that the best results are for the treatment (seed soaking + foliar spraying, as the length of roots (19.641) cm, and the share of plant (*P. hyberada*), on the other hand, the value of the third treatment in a plant (17.675) cm, the treatment (foliar spraying) ranks second in terms of impact and then the treatment of soaking only seeds, and distinguish the variety from the other variety.

Certainly on the one hand the effect of plant varieties outweigh the plant numerically and no significant because it carries the same characters from the other species in the same study. Whatever the best from the methods treatment was T₃ is meaning (seed soaking + foliar spraying), then second treatment (foliar spraying) and finally seed soaking .

4.2.3 Effect of SA on the Fresh Weight of Shoot

When we look at the data of the table (4.5), we find that there are no significant differences at the level of probability of 5% between most of the treatment and concentrations of salicylic acid, as the values took the same letters, and an indication of the absence of significant differences despite the presence of numerical differences

From the observation of table (4.5) concentration 100 ppm at the third treatment T₃ of the plant (*P. hyberada*), where (27.4) g, to the fresh weight of the shoot, while the lowest weight at (18.60) g plant (*P. niglefera*), clear the only field where significant differences are recorded at a level of probability of 5% is the interference among plants and the four SA concentrations, the highest shoot weight for plant at concentration is about 100 ppm (24.60) g and the lowest weight is (18.60) g, for plant (*P. niglefera*) when concentration was 300 ppm.

As for the other interventions and effects, whether for concentrations, varieties or transactions, there are no significant differences between them at the probability level of 5%, but there are numerical differences between them.

Table (4.5) Effect of salicylic acid concentrations and treatments on fresh weight of shoot (g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference plants × treatment	Effect of plants	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	18.72 7 a	19.867 a	19.66 a	16.55 a	18.60 a		
	T ₂	18.72 7 a	20.667 a	20.30 a	14.32 a	19.221 a		
	T ₃	18.72 7 a	20.80 a	20.66 a	13.50 a	21.065 a		
<i>P. hyberada</i>	T ₁	24.60 a	22.80 a	22.80 a	21.3 a	21.492 a		
	T ₂	24.60 a	26.80 a	24.60 a	21.28 3 a	22.967 a		
	T ₃	24.60 a	27.40 a	27.0 a	21.26 7 a	25.60 a		
Interference plant × Con.	<i>P. niglefera</i>	20.58 9 ab	22.161 ab	21.17 8 ab	13.50 b		19.771 a	
	<i>P. hyberada</i>	23.44 2 a	24.60 a	23.62 2 a	22.83 3 a		23.211 a	
Interference treatment × Con.	T ₁	19.98 3 a	22.042 a	21.03 3 a	19.26 3 a			19.833 a
	T ₂	19.98 3 a	22.95 a	22.33 a	19.05 a			21.094 a
	T ₃	19.98 3 a	27.2 a	25.60 a	19.05 a			23.546 a
Effect of Con.		20.88 3 a	23.138 a	22.89 2 a	19.05 a			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

4.2.4 Effect of SA on the Fresh Weight of Root

The results of table (4.6) shows that the treatment of a plant with salicylic acid at a concentration of 100 ppm and treatment T₃ (seed soaking + foliar spraying) we get the highest values as (7.667) g and there is significant differences from the concentrations and other treatments of the same plant (*P. hyberada*) or the other plant (*P. niglefera*). Plant root weights (*P. hyberada*) has the lowest value at 300 ppm (4.10) g, whereas in plant (*P. niglefera*) the best concentration is about 100 ppm (6.547) g, and the lowest value is at 300 ppm, reduction in plant root weight (3.941) g.

Table (4.6) Effect of salicylic acid concentrations and treatments on fresh weight of root (g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference plants × treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	3.667 bc	4.60 bc	4.60 bc	3.50 bc	3.941 b		
	T ₂	3.667 bc	5.50 ab	5.40 abc	1.20 c	4.066 b		
	T ₃	3.667 bc	6.547 ab	5.60 ab	1.20 c	5.265 ab		
<i>P. hyberada</i>	T ₁	5.28 abc	5.80 ab	5.683 ab	4.50 bc	4.461 ab		
	T ₂	5.28 abc	6.50 ab	6.467 ba	4.40 bc	5.386 ab		
	T ₃	5.28 abc	9.20 a	7.667 ab	4.10 bc	6.086 a		
Interference plant × Con.	<i>P. niglefera</i>	4.122 b	5.567 ab	5.149 ab	1.20 c		4.465 a	
	<i>P. hyberada</i>	5.28 ab	7.389 a	5.617 ab	4.622 b		5.271 a	
Interference treatment × Con.	T ₁	4.35 b	5.00 b	4.90 b	3.24 b			4.201 b
	T ₂	4.35 b	6.075 ab	5.10 b	2.98 b			4.666 ab
	T ₃	4.35 b	8.433 a	6.173 ab	2.76 b			5.736 a
Effect of Con.		4.372 bc	6.502 a	5.357 ab	3.24 c			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Interaction data between plant and salicylic acid concentrations shows that the concentration exceeded 100 ppm significantly at 5% probability level compared to other concentrations, especially in plant (*P. hyberada*) with root weight reaching (7.389) g, while increasing concentrations to 300 ppm leads to reduction in root weight value and reached its lowest value. In the plant (*P. niglefera*) by about (1.20) g.

In addition to the interaction data between the three treatments (seed soaking, foliar, foliar spraying, and seed soaking + foliar spraying) respectively, treatment T₃ (seed soaking + foliar spraying) at 100 ppm was found to have the best values and has significant differences at 5% probability level compared to other values. While the concentration of 300 ppm at the same treatment T₃ has the lowest values (2.76) g, we can say that the concentration of 100 ppm more influential in the plant (*Petunia* sp.), the plant recorded the highest response in all treatments, where the value of the roots of about (6.502) g.

Also, the interaction values between the plant and the three treatment methods, the results indicate that the T₃ treatment of the plant (*P. hyberada*) recorded the highest values by approximately (6.086) g, and gradually decreased the root weights of the plant to (3.941) g, and indicated the lowest value in the weight of the roots of the plant (*P. niglefera*), and there are significant differences between them at the probability level of 5%.

There are no significant differences in the effect of plants (*Petunia* sp) among them, although there are numerical differences between them, (5.271 and 4.465)g, respectively.

The effect of the treatments was evident from the significant differences between the results at the probability level of 5%. The third treatment T₃ recorded the highest effect on the weight of the roots of the plant approached (5.736) g compared to T₁ (seed soaking only) which is (4.201) g value.

4.3 Effect of SA on the Physiological Aspects:

4.3.1 Effect of SA on Water Content of Leaf Tissue

Note from the data of the table (4.9), are observed differences statistically significant for plant variety *P.hybrida* the highest values are treated for the third T₃ (183.28)% and then second treatment T₂ (183.28)% respectively for the same concentration which is 100 ppm while there are no significant differences at the level of probability of 5% between most of the coefficients and concentrations of salicylic acid as the values take the same letters, and an indication of the absence of significant differences despite the presence of numerical differences from the observation of table concentration 100 ppm at the third treatment T₃ of the plant (*P. niglefera*) is (157.26)%, to the water content of leaf tissue, while the lowest value (46.15)% at 300 ppm.

In addition to the interaction data between the three treatments (seed soaking, foliar spraying and seed soaking + foliar spraying) respectively and concentration of salicylic acid the highest values are found in the T₃ treatment called seed soaking and foliar spraying the plant where the highest value it is (170.20) %. followed by value (132.84)% then the value (157.33)% was for T₂ namely foliar spraying only. While the other values are not statistically significant below the probability level of

5% due to the similarity of the letters where they found the lowest numerical value (77.64)%.

Table (4.7) Effect of salicylic acid concentrations and treatments on water content of leaf tissue in water content (%) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species × treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	101.30 bcd	65.86 cd	94.54 bcd	46.15 d	73.07 b		
	T ₂	101.30 bcd	131.38 abc	149.26 ab	89.27 bcd	117.80 a		
	T ₃	101.30 bcd	157.26 ab	157.12 ab	89.27 bcd	126.24 a		
<i>P. hyberada</i>	T ₁	94.54 bcd	98.02 bcd	81.28 cd	91.86 bcd	79.33 b		
	T ₂	94.54 bcd	183.28 a	108.42 abcd	63.41c d	108.1 ab		
	T ₃	94.54 bcd	183.28 a	108.42 abcd	61.69 cd	108.1 ab		
Interference plant × Con.	<i>P. niglefera</i>	101.30 bc	116.73 abc	104.95 bc	80.65 cd		98.507 a	
	<i>P. hyberada</i>	46.15 d	149.28 a	124.13 ab	93.65 bc		105.703 a	
Interference treatment × Con.	T ₁	73.72 d	71.49 d	81.94 bcd	77.64 cd			76.20 b
	T ₂	73.72 d	157.33 a	128.84 abc	91.91 bcd			112.95 a
	T ₃	73.72 d	170.20 a	132.84 ab	91.91 bcd			117.17 a
Effect of Con.		73.73 b	133.0 a	114.54 a	87.15 b			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

While obviously the effects of SA concentrations on relative water content of leave found the highest statistically significant value at 100 ppm (133.0)% followed the value at 200 ppm (114.54)%.

The interaction between two species of plant and treatment shows no significant differences between three treatments of salicylic acid ,because the letters associated with the numbers are similar , but there are numerical differences the higher value (108.1)% and lower on (79.33)%,the results shows to the other variety T₃ have higher statically significant 126.24% and lower at (73.07)%.

The plant *P.hybrida* was superiorly significant, than other plant *P. niglefera* in terms of the impact of plant varieties. The value is (105.703)% and (98.507)% respectively

On the other hand, results of table (4.9) recorded significant differences using the three treatments on both plants despite the numerical differences between them. Values are gradually decreasing from the highest values (117.17)% to the lowest values at (76.20)%.

4.3.2 Effect of SA on Stability of Plasma Membranes

Seen through the table (4.10) that the stability of cytoplasmic membranes in plant effected by salicylic acid, therefore is found some different values that are statistically significant by salicylic acid concentrations and treatments, as for the first concentration 100 ppm (68.40)%, and the second concentration 200 ppm (66.67)%, have higher values compared with the concentration of control. While we note that the concentration of 300 ppm (27.366)% became less value than the control at (38.7)%, because the higher the concentration of salicylic acid a lower the stability of membranes this for the third treatment T₃, which is (seed soaking and foliar spraying), the same result as for seed soaking treatment and foliar spraying treatment this result share for *P. hybrida*.

As well as the other result for *P. hybrida* a three treatments showed that the membrane stability values were higher in concentration 100 ppm then 200 ppm, while the lowest values of concentration were at 300 ppm compared with the control. The lowest value was (25.50) %.

Data of table (4.10)% mentioned the interaction between three treatments and concentration the three coefficients showed different statistical changes where the highest values (53.55)% found in concentration 100 ppm, followed by the other two values (39.127, 32.916)% respectively.

However the effect of the four concentrations of SA, the concentration at 100 ppm had the highest values is (53.55)% compared to the control concentration, followed by a value (39.127 and 32.916) % of 200 and 300 ppm respectively.

Table (4.8) Effect of salicylic acid concentrations and treatments on Stability of plasma membranes (%) in leaf tissue of *Petunia sp.*

<i>Petunia sp.</i>	Treatment methods	Con. ppm				Interference species × treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	33.600 j	36.50 g	36.233 g	25.50 n	29.775 f		
	T ₂	33.600 j	40.466 d	37.466 f	25.50 n	35.333 e		
	T ₃	33.600 j	48.40 b	48.30 b	21.30 o	40.383 c		
<i>P. hyberada</i>	T ₁	38.70 e	43.466 d	40.83 d	32.533 k	38.041 d		
	T ₂	38.70 e	65.4 a	44.533 c	31.40 l	45.366 b		
	T ₃	38.70 e	68.40 a	66.67 a	27.366 m	50.333 a		
Interference plant × Con.	<i>P. niglefera</i>	31.433 g	38.70 c	36.355 d	31.544 g		34.38 3 b	
	<i>P. hyberada</i>	35.855 e	68.40 a	42.40 b	34.288 f		45.36 1 a	
Interference treatment × Con.	T ₁	35.883 e	44.433 b	40.516 d	34.416 f			35.079 c
	T ₂	35.883 e	50.11 a	42.883 c	33.983 g			40.35 b
	T ₃	35.883 e	53.55 a	51.32 a	32.916 h			44.187 a
Effect of Con.		33.894 c	53.55 a	39.127 b	32.916 d			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

So results of the interaction between *Petunia sp.* and methods treatment showed that the best results are for the treatment (seed soaking + foliar spraying), as (50.333)%, for the plant *P. hyberada* the lower value at (38.041)%, on the other side, the value of *P. niglefera* in the third treatment is (40.383)%, the treatment (foliar spraying) ranked second in terms of impact and then the treatment of soaking only seeds, and distinguish

Illustrated that effect of plants, the first class named *P. hyberada* the value is (45.361)% superior to the second class *P. niglefera* at (34.383)%.

The table finally confirms that the effect treatment there is significant differences between them at the probability level of 5%, the third treatment (seed soaking + foliar spraying), have highest value is (4.187)% superior to other treatments that were (40.35 and 35.079)% values respectively.

4.3.3 Effect of SA on the Permeability of Potassium (K^+)

The result of table (4.11) shows that the effects of salicylic acid is positive in enhancing and increasing the strength of plasma membrane by low infiltration and preserve the cell structure by low electrical conductivity value to indicate that the rate of cell membrane perfusion is little while the lowest concentration is found in the third treatment followed by the values compared with the control concentration.

Table (4.) Effect of salicylic acid concentrations and treatments on the permeability of K^+ (%) from the leaf tissue between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species × treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	212.56 7 a	40.567 h	80.667 e	98.9 c	120.988 a		
	T ₂	212.56 7 a	40.367 h	80.0 e	97.00 c	62.618 d		
	T ₃	212.56 7 a	40.267 h	81.4 e	98.9 c	72.075 c		
<i>P. hyberada</i>	T ₁	141.0 b	33.133 i	70.637 f	88.267 d	84.433 b		
	T ₂	141.0 b	31.483 i	70.567 f	86.43 d	50.192 e		
	T ₃	141.0 b	30.267 i	70.367 f	84.12 d	70.142 c		
Interference plant × Con.	<i>P. niglefera</i>	113.73 3 a	47.539 e	64.60 c	97.212 b		64.136 b	
	<i>P. hyberada</i>	98.90 b	36.844 f	58.10 d	97.0 b		89.346 a	
Interference treatment × Con.	T ₁	146.28 3 a	35.742 g	84.467 d	97.950 c			95.564 a
	T ₂	146.28 3 a	36.750 g	75.183 c	97.950 c			78.254 b
	T ₃	146.28 3 a	35.417 g	55.502 f	102.28 3 b			56.404 c
Effect of Con.		97.95 a	42.191 d	77.656 c	89.166 b			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

While the third concentration has high values compared with the control concentration this result is similar for all treatment for the class *P. hyberada* can be said that the lowest value of the concentration of 100 ppm is that (30.267)% the highest value of the concentration 300 ppm is (86.43)% in compare to control (141.0) % ,while the other result for *P. niglefera* the three treatment shows that the permeability of K^+ the lowest values are observed in the three treatments at 100 ppm concentration follow; p by 200 ppm concentration value , while the 300ppm

concentration shows the higher value (98.9)% followed by the second treatment value and then the first treatment comparison with the control (212.567)%.

The results of the interaction between plant and concentrations of salicylic acid shows there is obvious differences with statistical significance where the lowest values in plant variety are for concentration 100 ppm (36.844)% and a higher found in concentration 300 ppm (97.0)% this result share in *P. hybrida* , The results of the interference of the other class indicates that the lowest values are for 100 ppm concentration (47.539)% .The highest concentration values at 300 ppm is (97.212) % compared with the control (113.733)%.

Inferred from the results of the table that interaction between treatment and concentrations of salicylic acid appear that there are significant differences at the probability level 5%, between the values of concentrations and treatments, and the lowest value for T₃(35.417)% and the highest value(97.950)% for T₁ .

Also, the effect of salicylic acid concentrations show significant differences between them T₁ is the lowest value of the first concentration followed by the rise of the other concentrations compared to the control.

The interaction between the plant and the methods of treatment with salicylic acid and both plants are significant for other treatments.

Effect of salicylic acid on plants *Petunia* sp. and the extent of response to that effect, the plant (*P. hybrida*) significantly superior to the plant (*P. nigriflora*).

On the other hand, the effect of the treatments with salicylic acid there is significant differences the treatment T₃ occupied the lowest values (56.404)% followed by values upwards treatment T₂ (foliar spraying only) , and finally treatment T₁ (seed soaking only).

4.3.4 Effect of SA on the of Sodium Permeability of (Na⁺)

Table (4.12) appears that the effects of salicylic acid is positive in enhancing and increasing the strength of plasma membrane by low permeability and preserve the cell structure by low electrical conductivity value ,to indicate that the rate of cell membrane perfusion is little where the treatment showed the lowest values in

concentration 100 ppm and then the values escalated as the concentration increased compared to the control concentration where the lowest value (4.617)% is found in the third treatment highest (18.967)% in the first treatment of plant *P.hyberada*,

Table (4.10) Effect of salicylic acid concentrations and treatments on the permeability of Na⁺ (%) from the leaf tissue between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species × treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	77.637 a	11.567 def	15.367 de	39.667 c	46.901 a		
	T ₂	77.637 a	11.167 def	14.6 def	36.2 c	21.913 bc		
	T ₃	77.637 a	10.50 def	14.117 def	36.2 c	19.213 bc		
<i>P. hyberada</i>	T ₁	54.30 b	7.117 ef	12.567 def	18.967 d	23.613 b		
	T ₂	54.30 b	7.117 ef	12.0 def	17.467 de	20.496 bc		
	T ₃	54.30 b	4.617 f	11.637 def	16.567 de	17.708 c		
Interference plant × Con.	<i>P. niglefera</i>	54.3 a	11.911 d	14.383 d	33.773 b		19.139 b	
	<i>P. hyberada</i>	36.20 b	10.751 d	14.061 d	24.411 c		30.809 a	
Interference treatment × Con.	T ₁	45.25 a	11.402 c	14.733 c	27.992 b			33.698 a
	T ₂	45.25 a	10.617 c	14.067 c	14.983 c			20.66 b
	T ₃	45.25 a	8.592 c	14.00 c	27.992 b			20.563 b
Effect of Con.		45.25 a	11.331 d	19.236 c	24.078 b			

*Mean values followed by the same letter are not significantly different according to Duncan's multiple range test (P < 0.05).

Also note that the plant variety *P.niglefera*. demonstrated significant differences in values the less value (10.50%) % in concentration 100 ppm and the highest is (39.667)% interaction between the plant and three concentration shows that lowest value for interference of the *P. hyberada* plant variety is seen at 100 ppm (10.751)% and the higher value at 300 ppm is (24.411)%,the result of the second class showed that in concentration 100 ppm is low infiltration (11.911) % and in concentration 300ppm there is higher value in comparing to (33.773) % control.

In additionally to the interaction data between the three treatments (seed soaking, foliar spraying and seed soaking + foliar spraying) respectively and concentration of salicylic acid the lowest values were for concentration 100 ppm followed by values of 200 ppm and 300 ppm up wards the higher concentration value rose where the lowest value is (8.5)% for T₃and the highest was (27.992)% for T₁.

As for effect of four concentration of SA, the concentration of 100 ppm and are significantly different (11.331)%, followed by concentrations of 200 ppm at approximately (19.236) % and 300 ppm (24.078)%, respectively compared with the control value is (45.25)%.

Interaction between *Petunia* sp. And methods treatment showed that the best results were for the treatment (seed soaking + foliar spraying) was at (17.708)% and the share of plant *P. hyberada*, so that the value of the third treatment in a plant ,the treatment (foliar spraying) ranks second in terms of impact and then the treatment is (23.613)%of soaking only seeds, and distinguish the variety from the other variety.

While the preference was among the varieties of *petunia* plant for *P. hyberada* approximately (30.809) %, but *P. niglefera* was (19.139) %.

The effect of the treatments is evident from the significant differences between the results at the probability level of 5%. The third treatment T₃ recorded the lower effect on the permeability approached (20.563)% compared to T₁ (seed soaking only) which is higher (33.698) %, value.

4.3.5 Effect of SA on the Chlorophyll a Content

As can be seen from Table (4.13) The effect of salicylic acid on content of chlorophyll a of the *Petunia* sp. plants it can be seen from the number that the highest significant concentration is below the probability level 5% to the third treatment T₃ it is (2.46) mg / g for the concentration of 100 ppm and it is share of the plant *P. hybridia* . On the other hand there are no significant differences between them at the level of probability of 5%,because they take the same letters, but there is numerical differences. The same result related to *P. niglefera* it also have higher value in 100 ppm and for T₃ treatment which is (2.146) mg/g while the lowest concentration is at 100 ppm for the T₁ treatment it is (0.84) mg/g .On the other hand there are no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences.

Table (4.13) Effect of salicylic acid concentrations and treatments on chlorophyll a (mg/g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species × treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	0.55 c	0.84 c	1.06 bc	0.886 bc	0.861 b		
	T ₂	0.55 c	1.18 bc	1.086 bc	1.046 bc	0.993 b		
	T ₃	0.55 c	2.146 ab	1.45 abc	1.14 bc	1.349 ab		
<i>P. hyberada</i>	T ₁	0.66 c	1.326 abc	1.516 abc	1.346 abc	1.185 ab		
	T ₂	0.66 c	1.38 abc	1.82 abc	1.453 abc	1.295 ab		
	T ₃	0.66 c	2.46 a	1.82 abc	1.726 abc	1.639 a		
Interference plant × Con.	<i>P. niglefera</i>	0.55 c	1.388 ab	1.198 abc	1.0244 bc		1.068 b	
	<i>P. hyberada.</i>	0.66 c	1.862 a	1.596 ab	1.484 ab		1.373 a	
Interference treatment × Con.	T ₁	0.605 c	1.083 bc	1.288 bc	1.116 bc			1.023 b
	T ₂	0.605 c	1.49 b	1.27 bc	1.213 bc			1.144 ab
	T ₃	0.605 c	2.303 a	1.635 ab	1.433 bc			1.494 a
Effect of Con.		0.605 b	1.625 a	1.397 a	1.254 a			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Where the result of interference between plant and concentrations of salicylic acid shows that the concentration exceeded 100 ppm significantly at probability level 5% compared to other concentrations, especially in plant *P. hybrida* , (1.862) mg/g, While the values decreased significantly at the concentration of (200 and 300) ppm, respectively for the second class of plant *P. niglefera* , the highest value is 100 ppm (1.388) mg /g, and lower one at (1.0244) mg /g.

As for the interaction between treatment and concentration we notice that the third treatment (seed soaking + foliar spraying) has the highest values (2.303) mg/g especially at 100 ppm compared with other treatments and. Treatment Followed by the value of the concentration of 200 ppm (1.635) mg / g concentrations for the same treatment while the method of foliar spraying only has the highest value(1.49),(1.27) mg/g at the concentration of 100 ppm and then 200ppm respectively, while the lowest value is for seed soaking and its value (1.116) mg/g.

The effect of the four concentrations of SA watch that the three concentrate significantly different compare to control at probability level 5% the interference between *Petunia* sp. and methods treatment indicate a third treatment T₃ (seed soaking + foliar spraying) they have the highest values (1.639) mg/g wet weight show in genus *P. hyberada* while that the lowest value (0.861) mg /g wet weight show in *P. niglefera* at the concentration at 300 ppm.

The Interference between species and treatment show higher value (1.639) mg /g and lower at (1.185) mg /g That's for genus *P. hyberada*

The results for the other genus that the highest values for treatment (seed soaking + foliar spraying) it is (1.349) mg / g wet weight and lower on (0.861) mg/g for the T₁ (seed soaking).

Here, the data of the impact for the varieties, genus , was (1.373) mg /g is superior to *P. niglefera*, (1.068) mg / g.

At the latest on the effect of the treatments, the third treatment (seed soaking + foliar spraying) is significantly higher than the other treatments at 5% probability level. The top of the treatments with (1.494) mg /g value is superior to other treatments that it is (1.144 and 1.023) mg /g values respectively.

4.3.6 Effect of SA on the Chlorophyll b Content

The results indicated in the table (4.14) shows the effect of salicylic acid on chlorophyll b of the *Petunia* sp. Plants there are significant differences with different concentrations and treatments with salicylic acid of chlorophyll b content the highest value at 100 ppm concentration approximately (1.083) mg/g for the treatment T₃ (seed soaking + foliar spraying), the values belong to a plant *P. hyberada* as for the *P. niglefera*. The highest value of concentration is 100 ppm and for same treatment (0.913) mg/g and the lowest value for the same plant back to the concentration 100 ppm, but for T₁ treatment it is (0.4) mg/g.

Table (4.14) Effect of salicylic acid concentrations and treatments on chlorophyll b (mg/g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species× treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	0.55 bc	0.306 c	0.466 bc	0.4 bc	0.458 b		
	T ₂	0.55 bc	0.52 bc	0.486 bc	0.42 bc	0.521 b		
	T ₃	0.55 bc	0.913 ba	0.6 abc	0.5 bc	0.668 ab		
<i>P. hyberada</i>	T ₁	0.66 abc	0.54 bc	0.6 abc	0.546 abc	0.559 ab		
	T ₂	0.66 abc	0.76 abc	0.62 abc	0.586 abc	0.629 ab		
	T ₃	0.66 abc	1.083 a	0.78 abc	0.66 abc	0.768 a		
Interference plant × Con.	<i>P. niglefera</i>	0.55 ab	0.58 ab	0.517 ab	0.44 b		0.549 a	
	<i>P. hyberada</i>	0.66 ab	0.794 a	0.666 ab	0.597 ab		0.652 a	
Interference treatment × Con.	T ₁	0.605 b	0.423 b	0.553 b	0.473 b			0.508 b
	T ₂	0.605 b	0.64 b	0.553 b	0.503 b			0.575 ab
	T ₃	0.605 b	0.998 a	0.69 ab	0.58 b			0.718 a
Effect of Con.		0.605 a	0.687 a	0.592 a	0.518 a			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

The result of interference between plant and concentration of salicylic acid showed that the concentration exceeded 100 ppm significantly at probability level 5% compared to other concentrations specifically in genus (*P.hybrida*) plant approximately (0.794) mg/g the second highest value at (0.666) mg/g for the 200 ppm the other *P. niglefera* have highest value at 100 ppm (0.58) mg/g, while the lowest is (0.517) mg/g for the same concentration .

For the interaction between treatment and concentration the results suggest that the third treatment T₃ (seed soaking and foliar spraying) has the highest values (0.998) mg/g especially at 100 ppm in the chlorophyll a content compared with other treatments.

Also for the other methods the highest values are for concentration 100 ppm, while the lowest values are to the second concentration (200 and 300) ppm, respectively.

Effect of the four concentrations of salicylic acid, results shows that higher concentration related to 100 ppm it is (0.687) mg/g and the lowest (0.518) mg /g in the chlorophyll a content.

So interference between plants and treatments, note the superiority of one vegetation over the other in responding well to the effect of SA as well as in *P. hybrida* recorded the highest values for treatment (seed soaking + foliar spraying) it is (0.768) mg/g and lower (0.559) mg /g for the T₁ (seed soaking). As for second the plant *P. niglefera* it was the highest value for treatment (seed soaking + foliar spraying) approximately (0.668) mg /g, while the lower mg /g wet weight for the T₁ (seed soaking) method (0.458) mg /g.

Note the effecting of varieties the result shows that superiority *P. hybrida* (0.652) mg / g wet weight on the variety *P. niglefera* which values (0.549) mg /g.

Finally, on the impact of the treatments, the third treatment (seed soaking + foliar spraying) is significantly higher than the other treatments at 5% probability level the uppermost of the treatment with (0.718) mg / g value is superior to other treatment that is (0.575 and 0.508 mg / g) values respectively.

4.3.7 Effect of SA on the Chlorophyll a/b Content

Results are shows in the table (4.15) the effect of salicylic acid on chlorophyll a/ b of the *Petunia sp.* Plants there are high value (2.92) belong to genus *P.hybridia* for treatment T₃ (seed soaking + foliar spraying).

Note from the table that there are no significant differences between the rest of the numbers for different treatments and concentrations due to the similarity of letters, while there are numerical differences ,the less concentration in 100 ppm is (2.54) share in the transaction T₁(seed soaking),in the other hand the genus *P. niglefera* it clear from the results of the table that there are no significant differences between the different coefficients and concentrations due to the similarity of letters as there are numerical differences as the highest values were to treated T₃ (2.92) and the lower (2.34) in compare to control significantly at probability level 5% .

able (4.15) Effect concentrations of salicylic acid and treatments SA on chlorophyll a/b between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	0.66 b	2.34 ab	2.546 ab	2.46 ab	2.001 a		
	T ₂	0.66 b	2.686 ab	2.46 ab	2.64 ab	2.111 a		
	T ₃	0.66 b	2.78 ab	2.7 ab	2.666 ab	2.201 a		
<i>P. hyberada</i>	T ₁	0.55 b	2.54 ab	2.646 ab	2.58 ab	2.079 a		
	T ₂	0.55 b	2.66 ab	2.62 ab	2.62 ab	2.112 a		
	T ₃	0.55 b	2.92 a	2.736 ab	2.68 ab	2.221 a		
Interference plant × Con.	<i>P.hyberad a</i>	0.66 b	2.602 a	2.568 a	2.588 a		2.105 a	
	<i>P. niglefera.</i>	0.55 b	2.706 a	2.667 a	2.626 a		2.137 a	
Interference treatment × Con.	T ₁	0.605 b	2.44 a	2.596 a	2.52 a			2.04 a
	T ₂	0.605 b	2.673 a	2.54 a	2.63 a			2.112 a
	T ₃	0.605 b	2.85 a	2.718 a	2.673 a			2.211 a
Effect of Con.		0.605 b	2.654 a	2.618 a	2.607 a			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Interference effect between plant and concentration of salicylic acid shows that there is significant differences as the highest concentration of plant variety *P. hyberada* (2.706) at 100 ppm followed by a value of 200 ppm concentrations (2.667) the results are for *P. niglefera* the higher value (2.602) while the lower value (2.588) in compare to control significantly at probability level 5%.

The interaction between treatment and concentration, the treatment T₃ (seed soaking + foliar spraying), at 100 ppm (2.85) there is significant differences at probability level 5% followed by values (2.673, 2.44) respectively.

Illustrated the effect of the four concentrations of SA concentration of 100 ppm had the highest values (2.654) compared to the control concentration, followed by a concentration value (2.618 and 2.607) of 200 and 300 ppm respectively.

So results of the interaction between *Petunia* sp. and methods treatment shows that the best results are for the treatment (seed soaking + foliar spraying), as (2.221) for the plant *P. hyberada*, on the other hand, the value of *P. niglefera* in the third treatment is (2.201), the treatment (foliar spraying) ranks second in terms of impact

and then the treatment of soaking only seeds, and distinguish the variety from the other variety.

It turns out that effect of plants, the first class named *P. hyberada* has value (2.137) superior to the second *P. niglefera* the value as (2.105) .

The table finally confirms that the effect treatment there is no significant differences between them at the probability level 5%, but there are numerical differences between them.

4.3.8 Effect of SA on the Chlorophyll a+b Content

Inferred from the table shown in the table(4.16) the effect of salicylic acid on chlorophyll a+b of the *Petunia sp* plants .It can be seen from the number that the highest significant concentration is under the probability level 5% to the third treatment T₃ it is (3.56) mg / g for the concentration of 100 ppm And it was share of the plant *P. hybridia* the second value (2.58 and 2.386) mg / g belong to (200 and 300) ppm, respectably for the same treatment.

On the other hand there are no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences.

The result for *P. niglefera* it also has higher value in 100 ppm and for T₃ treatment which is (3.106) mg/g, however there is no significant differences between them at the level of probability of 5% because they took the same letters, but there are numerical differences.

Interaction between SA concentrations and plants, the concentration of 100 ppm in the *P. hyberada* plants took the highest values and differed significantly from the rest of the concentrations it is (2.673) mg/g, in another side lowest value is (13.60) mg/g in the *P. niglefera* with 300 ppm SA concentration and treatment method seed soaking + foliar spraying T₃.

Table (4.16) Effect concentrations of salicylic acid and treatments on chlorophyll a+b (mg/g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species× treatment	Effect of species	Effect of treatment	
		0	100	200	300				
<i>P. niglefera</i>	T ₁	0.66 de	0.96 cde	1.56 bcde	1.28 dce	1.115 b			
	T ₂	0.66 de	1.646 bcde	1.566 bcde	1.56 bcde	1.358 b			
	T ₃	0.66 de	3.106 ab	1.94 abcde	1.6 bcde	1.826 ab			
<i>P. hyberada</i>	T ₁	0.55 e	1.88 abcde	1.986 abcde	1.946 abcde	1.59 ab			
	T ₂	0.55 e	2.58 abc	2.04 abcde	1.96 abcde	1.782 ab			
	T ₃	0.55 e	3.56 a	2.58 abc	2.386 abcd	2.269 a			
Interference plant × Con.	<i>P. niglefera</i>	0.66 cd	1.904 ab	1.688 b	1.48 bc		1.433 b		
	<i>P. hyberada</i>	0.55 d	2.673 a	2.202 ab	2.097 ab		1.88 a		
Interference treatment × Con.	T ₁	0.605 c	1.42 bc	1.773 bc	1.613 bc				1.352 b
	T ₂	0.605 c	2.113 b	1.803 bc	1.76 bc				1.57 ab
	T ₃	0.605 c	3.333 a	2.26 b	1.993 b				2.047 a
Effect of Con.		0.605 b	2.288 a	1.945 a	1.788 a				

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

So results of the interaction between the treatment methods and SA concentrations shows a significant superiority of the third treatment, namely, (seed soaking and foliar spraying), especially at 100 ppm compared with other treatments and concentration, ranks second in terms of effect the concentration of 200 ppm, if the values (3.333 and 2.26) mg/g the 100 ppm by foliar spraying only ranks third in terms of the high value number that was (2.11) mg/g the lowest values are for the concentration of 100 ppm which became (1.42) mg/g which it significantly different even from the control treatment it is (0.605) mg/g .

As for the effect of the four concentrations of salicylic acid, the concentrations of 100 ppm, 200 ppm and 300ppm were significantly different, as (2.288 and 1.945) mg / g respectively, while the lowest rise is (1.788) mg /g at 300 ppm.

Concerning the interference between *Petunia sp.* And methods treatment a third treatment (seed soaking + foliar spraying) outperformed the rest of the treatments, especially the *P. hyberada* where the highest value reaches (2.26), mg/g while the lowest value at the concentration 300 ppm treatment of *P. niglefera* it is (1.115) mg /g .

While the preference among the *Petunia sp.* plants for *P.hyberada* approximately (1.88) mg / g but *P. niglefera* is (1.433) mg / g.

Finally, on the effect of the treatments, the third treatment (seed soaking + foliar spraying) is significantly higher than the other treatments at 5% probability level. The top of the treatment with (2.047) mg/g value is superior to other (1.57 and 1.352) mg /g values respectively .

4.3.9 Effect of SA on the Carotene Content

The data of the table (4.17) shows activity of salicylic acid in a significant increase in the carotene content of the plant for the *Petunia sp.* It can be seen from the values that the highest significant concentration is below the probability level 5% to the third treatment T₃ it is (9.63) mg/g for the concentration of 100 ppm and it is share of the plant *P. hyberada*, while the same concentration, for the same treatment but for plant *P. niglefera* is (6.98) mg /g. on the other hand there are no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences..

Table (4.15) Effect of salicylic acid concentrations and treatments on carotene (mg/g) between the two species.

Petunia sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment		
		0	100	200	300					
P. niglefera	T ₁	2.55 b	6.033 ab	6.00 ab	3.81 b	4.01 a				
	T ₂	2.55 b	6.487 ab	6.033 ab	4.853 ab	5.236 a				
	T ₃	2.55 b	6.98 ab	6.05 ab	3.907 b	5.706 a				
P. hyberada	T ₁	3.47 b	6.807 ab	6.507 ab	5.887 ab	5.181 a				
	T ₂	3.47 b	7.42 ab	6.867 ab	5.187 ab	5.96 a				
	T ₃	3.47 b	9.63 a	7.003 ab	5.45 ab	6.15 a				
Interference plant × Con.	P. niglefera	2.55 c	6.491 a	6.451 a	4.848 bc		5.113 a			
	P. hyberada	3.47 bc	6.911 a	6.623 a	5.646 ab		5.633 a			
Interference treatment × Con.	T ₁	3.01 b	6.42 ab	5.837 ab	4.953 ab					4.622 a
	T ₂	3.01 b	6.507 ab	6.158 ab	5.68 ab					5.443 a
	T ₃	3.01 b	8.317 a	6.735 ab	4.848 ab					6.055 a
Effect of Con.		3.01 b	6.557 a	6.278 a	5.649a					

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

The lowest carotene of the plant is recorded at the concentration of 300 ppm in T₃ treatment and it was the plant share *P. niglefera* it was (3.81) mg / g, while at the same concentration and the same treatment of plant *P. hyberada* weight value (5.887) mg / g.

Regarding the interaction between *Petunia* sp. and salicylic acid concentrations, we obtain the highest value of carotene content at 100 ppm (6.911 and 6.491) mg / g for *P. hyberada* and *P. niglefera* respectively, and there are no significant differences at 5% probability level between the two values because they couple the same letters, but this does not prevent numerical differences between them. There are also significant differences between the values due to the difference of the characters associated with the numbers. The lowest value is recorded at the concentration of 300 ppm for *P. niglefera* is (4.848) mg / g.

As for the interaction between the concentrations of salicylic acid and the treatment methods, the best values recorded will be found at the concentration of 100 ppm in treatment T₃ (8.317) mg / g and then the concentration of 200 ppm, (6.735) mg / g weight for the same treatment, then the second treatment T₂ (foliar spraying) at concentration 100 ppm. For the same table note that there are significant differences at the level of probability of 5% between the concentrations and treatments with salicylic acid, and the lowest values are recorded at the concentration of 300 ppm (4.953) mg / g, and this value is the lowest compared to the control treatment, as (3.01) mg / g .

When we look at the effects of SA concentrations on carotene content of plant were observed significantly at the 5% probability level among all four concentrations, higher was at 100 ppm(6.557) mg /g while lower value show at 300 ppm it is (5.649) mg / g .

The data of the Interaction between plant and salicylic acid treatments showed no significant differences between plants (*P. hyberada* and *P. niglefera*) respectively in treatment T₃ because the characters associated with the numbers are identical, but there are numerical differences (6.15 and 5.706) mg / g .

Also we can see that there were significant differences between the three treatments with salicylic acid treatment T₃ occupied the highest values of carotene and then treatment T₂ (foliar spraying only), and finally treatment T₁ (seed soaking only).

Effect of salicylic acid on plants *Petunia* sp. there is no significant differences in the among them, although there were numerical differences between them (5.113and5.633) mg/g respectively.

4.3.10 Effect of Salicylic Acid on Carbohydrate Content

Results indicated in table (4.18) effect of salicylic acid on the content carbohydrates of *Petunia* sp. plants. The highest values are significant for the carbohydrate concentration of the plant variety *P.hyprida* in the third treatment T₃ (88.33) mg/g at SA concentration 100 ppm, followed by the second highest value for the same parameter, but the concentration is 200 ppm (78.67) mg/g. The highest

values were also found for the other plant variety *P. niglofrea* in the third treatment T_3 (seed soaking + foliar spraying) of concentration 100 ppm at (88.00)mg/g, and 200 ppm at (62.00) mg/g, respectively. On the other hand there are no significant differences between them at the level of probability of 5% , because they took the same letters, but there are numerical differences.

Table (4.18) Effect of salicylic acid concentrations and treatments on carbohydrate content (mg / g dry weight) in leaf tissue between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T_1	34.00 d	58.00 cd	48.0 cd	44.00 cd	43.50 c		
	T_2	34.00 d	61.33 bcd	60.67 bcd	44.00 cd	48.5 bc		
	T_3	34.00 d	88.00 a	62.00 bc	43.00 cd	56.25 bc		
<i>P. hyberada</i>	T_1	42.00 cd	60.00 bcd	50.67 cd	47.00 cd	43.50 c		
	T_2	42.00 cd	62.00 bc	61.00 bcd	47.50 cd	53.667 b		
	T_3	42.00 cd	88.33 a	78.67 ab	46.00 cd	72.250 a		
Interference plant × Con.	<i>P. niglefera</i>	34.0 d	67.0 a	52.333 bc	46.667 cd		47.750 b	
	<i>P. hyberada</i>	42.0 cd	70.0 a	61.55 ab	50.00 bc		58.139 a	
Interference treatment × Con.	T_1	38.0 c	59.333 ba	59.0 ba	44.0 bc			46.0 b
	T_2	38.0 c	62.0 ab	60.833 ab	47.333 bc			54.958 a
	T_3	38.0 c	68.0 a	66.167 a	54.667a bc			57.875 a
Effect of Con.		38.00 b	61.167 a	58.50 a	54.11 a			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

The results of the table also confirm a significant interference below the probability level 5% for the effect of interference between the plant and the concentration of salicylic acid, the plant has highest value (70.0) mg/g and (61.55) mg/g respectively in compeer to control share in *P. hybrida* ,the second plant variety *P. niglofera* share the high value at 100 ppm (67.0) mg/g, and (52.333) mg/g at 200 ppm. On the other side there is no significant differences between them at the level of probability of 5% , because they took the same letters, but there are numerical differences. The lower value it is (46.667) mg/g high value at 100 ppm(67.0) mg/g, and(52.333) mg/g at 200 ppm. On the other side there are no significant differences between them at the level of probability of 5% , because they took the same letters, but there are numerical differences. The lower value it is (46.667) mg/g.

Additionally the results of the interaction between the treatment methods and SA concentrations. The results shows that the highest values is significant different for interference is (68.0) at 100 ppm, but there are no statistically significant differences for other value where the lowest values at (44.0)mg/g.

As for the effect of the four concentrations of SA, the concentrations of 100 ppm and significantly different in value is (61.167) mg/g, followed by concentrations of 200 ppm at (958.50) mg/g, and 300 ppm (54.11) mg/g, respectively, compared with the control value is (38.00) mg/g.

Interaction between *Petunia* sp. Plants, and methods treatment the third treatment (seed soaking + foliar spraying) results indicate that the T₃ treatment of the plant *P. Hyberada* recorded the highest values by approximately (72.250) mg/g, and gradually decreased the carbohydrates concentration of the plant to (43.50) mg/g, and indicated the lowest value (43.50) mg/g in the concentration of carbohydrate of the plant *P. niglefera*,, and there is significant differences between them at the probability level of 5%..

There were a significant differences in the effect of plant *Petunia* sp. in carbohydrate content, the genus *P. hyberada* (58.139) mg/g significantly superior to the plant *P. niglefera* at (47.750) mg/g.

At list on the effect of treatment the best from the methods treatment is T₃ is meaning (seed soaking + foliar spraying), then second treatment (foliar spraying) and finally seed soaking .

4.3.11 Effects of Salicylic Acid on Na⁺ Content of *Petunia* sp. Plant

Table (4.19) shows a significant increase in Na⁺ concentration in total shoot of *Petunia* sp. the highest concentration of Na⁺ is found in the third treatment T₃ it is (3.833) mg / g for the concentration of 100 ppm, and it is share of the plant variety *P. hyberada* the second value founded in the same treatment, but for 200 ppm it is (3.50) mg / g, on the other hand there are no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences.

Table (4.17) Effect of salicylic acid concentrations and treatments on concentration of Na⁺ (mg / g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment	
		0	100	200	300				
<i>P. niglefera</i>	T ₁	1.20 dc	1.54 cd	1.45 cd	1.050 d	1.187 b			
	T ₂	1.20 dc	2.40 abcd	1.90 abcd	1.050 d	1.394 b			
	T ₃	1.20 dc	3.20 abc	1.60 cd	1.050 d	1.575 b			
<i>P. hyberada</i>	T ₁	1.40 cd	1.90 abcd	1.80 bcd	1.326 cd	1.399 b			
	T ₂	1.40 cd	2.86 abcd	2.40 abcd	1.30 cd	2.508 a			
	T ₃	1.40 cd	3.833 a	3.50 ab	1.281 cd	2.75 a			
Interference plant × Con.	<i>P. niglefera</i>	1.393 bc	2.40 ab	1.566 bc	1.05 c		1.327 b		
	<i>P. hyberada</i>	1.533 bc	2.811 a	2.366 ab	1.297 bc		2.277 a		
Interference treatment × Con.	T ₁	1.725 ab	1.633 ab	1.40 ab	1.15 b				1.381 b
	T ₂	1.725 ab	2.39 ab	2.1 ab	1.25 ab				1.951 ab
	T ₃	1.725 ab	2.616 a	2.55 ab	1.363 ab				2.074 a
Effect of Con.		1.725 ab	2.188 a	1.88 ab	1.415 b				

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test (P < 0.05).

Notes from the same table (4.19) the result of *P.nigloferia* there is no significant differences between them at the level of probability of 5% because they took the same letters, but there is numerical differences the highest concentration of Na^+ is found in the third treatment T_3 it is (3.833) mg / g dry weight at 100 ppm , while the lower concentration it is , (1.050) mg / g dry weight at 300 ppm.

Have been observed the interaction between SA concentrations and plant varieties indicates there is no significant differences between the highest values of both plants (2.811 and 2.40) mg / g dry weight, although there are numerical differences at the best concentration 100 ppm, also the lowest Na^+ concentration in a plant, which was at a concentration of 300 ppm approximately (1.05) mg / g dry weight.

Effect of Interference between the treatment and concentrations of salicylic acid , the values are significantly different from one another because non-similar characters that accompany the numbers likewise for the *P. hybrida* the highest value of interference is (2.616) mg / g dry weight , at a concentration of 100 ppm, and the lowest value is (1.399) mg / g dry weight for the control treatment, the other species the highest value of interference was (1.575) mg /g dry weight , at a concentration of 100 ppm, and the lowest value is (1.187) mg / g dry weight for the control treatment.

While the four concentrations of SA show no significant differences between them at the level of probability 5% because they take the same letters, but there is numerical differences the highest value for 100 ppm it is (2.188) mg / g dry weight and the lower one (1.415) mg / g dry weight at 300 ppm.

Shows the results mentioned in the table the interference between *Petunia sp.* and methods treatment that the treatment (seed soaking + foliar spraying) T_3 has the top value it is (2.75) mg / g dry weight share for *P.hyberada* while the lower at T_1 treatment (1.399) mg / g dry weight, in the other hand the species *P.niglofera* have also higher value for T_3 treatment it was (1.575) mg / g dry weight, and lower for T_1 it is (1.050) mg / g dry weight. Interference among the varieties of petunia plant observe for *P.hyberada* approximately (2.277) mg / g dry weight, but *P. niglefera* is (2.277) mg / g dry weight.

Inferred from the last field in the table that the effect of the treatments, the third treatment T_3 (seed soaking + foliar spraying) was significantly higher than the other treatments at 5% probability level. The top of the treatment with (2.074) mg/g dry weight value was superior to other transactions that were (1.951 and 1.381) mg / g dry weight values respectively.

4.3.12 Effect of Salicylic Acid on Ca^{+2} Content of *Petunia* sp. Plant

Table (4.20) shows treatment with salicylic acid has affected on Ca^{++} concentration the third treatment T_3 It has given highest value (10.5) mg / g at 100 ppm share in plant variety *P. hyberada* in the same treatment for the variety *P. niglefera* the lower value belong to t_1 named seed soaking it is (5.2 and 3.1) mg / g for variety *p. hyberada* ,and *P. niglefera* respectively moreover, there are no significant differences between them at the level of probability of 5% because they take the same letters, but there is numerical different.

As for the effect of interaction between salicylic acid concentrations and plant varieties the top Concentrations appeared at 100 ppm and 200 ppm (7.967 and 8.5) mg / g respectively it is observed in treatment T_3 (seed soaking + foliar spraying), in the other hand the result Indicates there are no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences while lowest value share in, *P. niglefera* is (4.561) mg / g for 300 ppm concentration.

Interaction data among three treatments (seed soaking, foliar spraying and seed soaking + foliar spraying) respectively and salicylic acid concentrations showed treatment T_3 (seed soaking + foliar spraying) at 100 ppm is found to have the best values and had significant differences at 5% probability level compared to other values while the concentration of 300 ppm at the same treatment T_3 has the lowest values (5.183) mg / g, we can say that the concentration of 100 ppm more influential in the plant *Petunia* sp., the plant recorded the highest response in all treatments, where the value of the Ca^{+2} about (8.50) mg / g.

Table (4.20) Effect of salicylic acid concentrations and treatments on concentration of Ca^{++} (mg / g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment	
		0	100	200	300				
<i>P. niglefera</i>	T ₁	3.6 bc	1.4 c	4.2 bc	3.1 bc	3.075 b			
	T ₂	3.6 bc	5.1 bc	4.767 bc	5.383 abc	4.713 ab			
	T ₃	3.6 bc	6.5 abc	6.5 abc	5.2 bc	5.45 ab			
<i>P. hyberada</i>	T ₁	4.4 bc	2.9 c	8.5 ab	5.2 bc	5.25 ab			
	T ₂	4.4 bc	10.5 a	8.5 ab	5.2 bc	7.15 a			
	T ₃	4.4 bc	10.5 a	8.5 ab	5.167 bc	7.142 a			
Interference plant × Con.	<i>P. niglefera</i>	3.6 b	4.333 b	5.156 b	4.561 b		4.412 b		
	<i>P. hyberada</i>	4.4 b	7.967 a	8.5 a	5.189 b		6.513 a		
Interference treatment × Con.	T ₁	4.00 bc	2.15 c	6.35 ab	4.15 bc				4.162 b
	T ₂	4.00 bc	7.8 a	6.633 ab	5.292 abc				5.931 a
	T ₃	4.00 bc	8.50 a	7.5 ab	5.183 abc				6.295 a
Effect of Con.		4.00 c	6.827 a	6.15 ab	4.875 bc				

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

For the effect of concentrations the highest value is statistically significant at the level of probability of 5% at concentration 100 ppm (6.827) mg / g.

Also the interaction values between the plant and the three treatment methods, the results indicate that the T₃ treatment of the plant (*P. hyberada*) recorded the highest values by approximately (7.142) mg/g, and gradually decreased the SA concentration of the plant to (5.25) mg/g, and indicated the lowest value in the weight of the SA of the plant (*P. niglefera*), and there are Significant differences between them at the probability level of 5%. There is significant differences in the effect of plants (*Petunia* sp), (*P. hyberada*) is superior (6.513) mg/g, in compare with (*P. niglefera*) (4.412) mg /g.

The effect of the treatments is evident from the significant differences between the results at the probability level of 5%. The third treatment T_3 records the highest effect on the weight of the roots of the plant a proached (6.295) mg/g, compared to T_1 (seed soaking only) which was (4.162) mg/g, value, the result related to *P. niglefera* there is no significant differences between them at the level of probability of 5% because they take the same letters, but there is numerical differences the higher value at 100 ppm for T_3 it is (6.5) mg/g and the lower one at 300 ppm for (1.4) mg/g for the same concentration but for T_1 .

4.3.13 Effect of Salicylic Acid on Nitrogen N^+ Content of *Petunia* sp. Plant

Table (4.19) Effect of salicylic acid concentrations and treatments on concentration of nitrogen (mg / g) between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	2.100 c	3.107 c	3.09 c	1.16 c	1.133 c		
	T ₂	2.100 c	4.42 bc	3.13 c	1.15 c	3.320 ba		
	T ₃	2.100 c	4.82 bc	4.817 bc	1.14 c	3.707 ab		
<i>P. hyberada</i>	T ₁	2.918 c	3.95 bc	3.84 bc	2.77 c	1.868 bc		
	T ₂	2.918 c	4.42 bc	4.42 bc	2.12 c	3.829 ab		
	T ₃	2.918 c	10.357 a	8.5 ab	1.468 c	5.356 a		
Interference plant × Con.	<i>P. niglefera</i>	2.10 bc	4.42 ab	3.176 bc	1.239 c		3.005 a	
	<i>P. hyberada</i>	2.407 bc	5.766 a	4.489 ab	2.019 bc		3.398 a	
Interference treatment × Con.	T ₁	3.098 bcd	3.26 bcd	3.11 bcd	2.445 cd			
	T ₂	3.098 bcd	4.383 abc	4.33 abc	1.639 cd	3.767 a		
	T ₃	3.098 bcd	6.637 a	5.815 ab	1.43 d	4.337 a		
Effect of Con.		3.26 ab	4.47 a	3.447 a	1.629 b			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

The results of table (4.21) shows the effects of salicylic acid on the nitrogen content of the *Petunia* sp., for most concentrations positive compare to the control treatment, there is certainly significant differences at the level of probability 5%, the highest value g, at a concentration of 100 ppm in treatment The third T₃ for the plant *P. hyberada* (10.357) mg / g, followed by the same plant and the same treatment, but at a concentration of 200 ppm (8.5) mg / g, on the other hand there are no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences For *P. niglefera* the lowest concentration at 300 ppm for the T₁ treatment it was (1.16) mg / g.

The interaction between plant and SA concentrations shows that the concentration of 100 ppm in the plant (*P. hyberada*) at the highest value (5.766) mg/ g, while the lowest values at the concentration of 300 ppm (1.239) mg/ g ,for plant *P. niglefera*, is higher compared to the control treatment.

The best results in increasing nitrogen of plant shoot are (6.637) mg/g, and there is no significant differences at the probability level 5%, between the values of concentrations and treatments but there are numerical differences, the lowest value for nitrogen (2.445) mg/g. however, compared to the value of the comparison transaction.

Also, the effect of salicylic acid concentrations without significant differences between the four concentrations on the increase of nitrogen of plant shoot , the highest value (4.47) mg/ g at 100 ppm and (1.629) mg/g at 300 ppm concentration which is higher compared to control treatment (3.26) mg/g.

The interaction between the plant and the methods of treatment with salicylic acid and both plants are significant for other treatments.

While the effects of the response to the treatment with salicylic acid with *P. hyberada* plant is more positive compared to the response to *P. niglefera* and in terms of different characters associated with the figures.

On the other hand, we find significant differences using the three treatments on both plants the higher and lower value (4.337and 1.50) mg/g respectively.

There were no significant differences in the effect of plants *Petunia* sp among them, although there were numerical differences between them, (3.398 and 3.005) mg/g, respectively.

4.3.14 Effect of Salicylic Acid on phosphate(P^{2-}) Content of *Petunia* sp. Plant

The table(4.22) illustrates the effect of SA on concentration of phosphate in the vegetative group of plant there are significant increase phosphate at 100 ppm for treatment T_3 (seed soaking + foliar spraying) the higher value share in *P.hybrada* (1.060) mg/g , on the other hand there are no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences for *P. niglefera* the lowest concentration at 300 ppm for the T_1 treatment it was (0.42) mg / g

Table (4.20) Effect of salicylic acid concentrations and treatments on concentration of phosphate (mg / g) between the two species.

of phosphate (mg / g) between the two species.									
Petunia sp.	Treatment methods	Con. ppm				Interference species ×treatment	Effect of species	Effect of treatment	
		0	100	200	300				
P. niglefera	T ₁	0.52 b	0.60 ab	0.54 b	0.42 b	0.392 b			
	T ₂	0.52 b	0.620 ab	0.56 b	0.410 b	0.530 ab			
	T ₃	0.52 b	0.72 ab	0.60 ab	0.246 b	0.584 ab			
P. hyberada	T ₁	0.550 b	0.62 ab	0.56 b	0.43 b	0.507 ab			
	T ₂	0.550 b	0.626 ab	0.564 b	0.45 b	0.537 ab			
	T ₃	0.550 b	1.060 a	0.60 ab	0.366 b	0.732 a			
Interference plant × Con.	P. niglefera	0.528 a	0.640 a	0.573 a	0.422 a		0.479 b		
	P. hyberada	0.550 a	0.698 a	0.573 a	0.450 a		0.6155 a		
Interference treatment × Con.	T ₁	0.50 ab	0.56 ab	0.553 ab	b				0.461 b
	T ₂	0.50 ab	0.593 ab	0.56 ab	0.388 b				0.545 ab
	T ₃	0.50 ab	0.840 a	0.64 ab	0.426 b				0.635 a
Effect of Con.		0.500 a	0.607a	0.584 a	0.497 a				

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Effect of interaction between plant variety and different concentration of salicylic acid show there is no significant differences between them at the level of probability of 5% because they take the same letters, but there are numerical differences the higher value (0.698) mg/g share in *P.hybrada* while the lower (0.422) mg/g share in *P. niglofera* .

As note the interference between three treatment and different concentration there is no significant differences at the probability level 5%, between the values of concentrations and treatments but there are numerical differences the lowest value for phosphate (0.403) mg/g and the higher one (0.840) mg/g in compeer to control while the effect of four concentration of salicylic acid on plat with no significant differences the concentration 100 ppm take the value (0.607) mg/g, the concentration 300 ppm take lower value (0.497) mg/g.

For the effect of interaction between plant varieties and the three treatments there is significant differences in the effect of plants (*Petunia sp.*) the genus *P.hybrada* (.6155) mg/ g wet weight is superior to the variety *P. niglefera* (0.479) mg/g fresh weight.

The results showed that the effect of interaction between plant varieties and the three treatments plant varieties exhorted the highest values in the third treatment (0.732, 0.584) mg/g for *P.hybtida* and *P. niglofera* respectively.

The latest on the effect of the treatments, the third treatment (seed soaking + foliar spraying) is significantly higher than the other treatments at 5% probability level. The top of the treatment with (0.635) mg/g value is superior to other transactions that are (0.545 and 0.461) mg / g values respectively.

4.3.15 Effect of Salicylic Acid on K⁺ Content of *Petunia sp.* Plant

The results indicate from the table (4.23) the effect of salicylic acid on Potassium of the *Petunia sp* plants ,it can be seen from the number that no significant different below the probability level 5 % because they take the same letters, but there are numerical differences the higher number to the treatment T₃ (5.00) mg / g for the concentration of 100 ppm and the lower (1.70) mg/g and it is share of the plant variety *P. hybridia* ,on the other hand the plant variety *P. niglefera* have higher significant number at 100 ppm related to third treatment T₃ seed soaking and foliar spraying (5.00) mg/g .

Table (4.21) Effect of salicylic acid concentrations and treatments on concentration of K⁺ (mg / g) between the two species..

<i>Petunia sp</i>	Treatment methods	Con. ppm				Interference species × treatment	Effect of species	Effect of treatment
		0	100	200	300			
<i>P. niglefera</i>	T ₁	2.20 cd	3.27 abc	2.600 bcd	1.66 cd	1.50 c		
	T ₂	2.20 cd	3.300 abc	3.00 abcd	1.60 cd	1.966 c		
	T ₃	2.20 cd	4.50 ab	3.14 abcd	1.300 d	3.091 ab		
<i>P.hyberada</i>	T ₁	2.90 abc	3.44 abc	2.600 bcd	1.70 cd	1.50 c		
	T ₂	2.90 abc	3.666 abc	3.20 abcd	1.70 cd	2.40 bc		
	T ₃	2.90 abc	5.00 a	3.24 abcd	1.500 cd	3.925 a		
Interference plant × Con.	<i>P. niglefer</i>	1.588 c	3.322 a	2.70 ab	1.50 c		1.655 b	
	<i>P.hyberada</i>	1.966 c	3.633 a	2.90 ab	1.566 c		3.138 a	
Interference treatment × Con.	T ₁	2.20 ab	2.450 ab	2.40 ab	1.30 b			1.950 b
	T ₂	2.20 ab	2.900 a	2.633 ab	1.900 ab			2.295 ab
	T ₃	2.20 ab	3.40 a	3.083 a	2.00 ab			2.945 a
Effect of Con.		2.433 a	2.611 a	2.444 a	2.100 a			

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test (P < 0.05).

It is evident from the interaction between plants and treatments that there are significant differences between the concentration 100 ppm and 200 ppm in compeer respectively in compere to control for the two plants varieties.

While overlapping effect between three treatment (seed soaking , foliar spraying and seed soaking&foliar spraying) without significant different below the probability level 5 % because they take the same letters, but there are numerical differences the higher number to the treatment T₃ (3.40) mg/g, while the lower on it is (1.30) mg/g.

The four concentrations of acid illustrate their effect without significant different below the probability level 5% because they took the same letters, but there are numerical differences the higher for 100 ppm followed (200and300) ppm respectively .

As for the interaction between species and treatment the results showed that the plant variety (*P. hybrida*) the highest values for treatment (seed soaking + foliar spraying) it is (3.925) mg / g wet weight and lower on (1.50) mg / g for the T₁ (seed soaking). As for second the plant variety (*P. niglefera*) it is the highest value for treatment (seed soaking + foliar spraying) (3.091) mg / g, while the lower (1.50) mg / g for the T₁ (seed soaking)method .

Here, the results of the impact of the varieties the genus *P.hybrada* (3.138) mg /g is superior to the variety *P. niglefera* (1.655) mg / g .

Effect of salicylic acid on plants *Petunia sp.* there is significant differences in the effect of plants (*Petunia sp*) the higher and lower it is (2.945 and1.950) mg/ g, respectively.

4.3.16 Effect of Salicylic Acid on Protein Content

The results explained in the table (4.24) shows effect of salicylic acid on protein content for *Petunia sp.* the concentrations of SA increase significant at treatment T₃ is meaning (soaking deed + foliar spraying) at 100 ppm (64.73) mg/g. while the second concentration has the second highest value for the T₂ treatment the value is (30.13) mg/g these result share for *P.hybirida* .On the other hand there is no significant differences between them at the level of probability of 5%, because they

take the same letters, but there is numerical differences .while other genus *P.niglifera* the highest value it was also for T₃ and at 100 ppm is (53.13) mg/g.

As for the least values, although there is no statistical significance because of the similarity of characters, but there is a numerical change the values of the first plant *P.hybridia* (13.13) mg/g the other *P. niglefera* was (8.8) mg/g at 300 ppm.

The results of the interaction between plant and concentrations of salicylic acid shows that the plant variety *P. hybridia* had the highest concentration at 100 ppm (36.037) mg / g the other higher value related to *P. niglefera* is (28.058) mg/g.

Table (4.24) Effect of salicylic acid concentrations and treatments on the content of proteins (mg/g) in the leaf tissue between the two species.

<i>Petunia</i> sp.	Treatment methods	Con. ppm				Interference species× treatment	Effect of species	Effect of treatment		
		0	100	200	300					
<i>P. niglefera</i>	T ₁	19.56 c	13.25 c	13.13 c	8.8 c	7.081 c				
	T ₂	19.56 c	24.00 bc	17.31 c	8.8 c	20.766 ab				
	T ₃	19.56 c	53.13 ab	24.69 bc	9.54 c	23.934 ab				
<i>P. hyberada</i>	T ₁	27.63 bc	18.31 c	13.13 c	13.13 c	11.674 bc				
	T ₂	27.63 bc	30.11 ab	17.56 c	10.25 c	23.169 ab				
	T ₃	27.63 bc	64.73 a	30.13 ab	11.23 c	7.751 a				
Interference plant × Con.	<i>P. niglefera</i>	13.13 bc	28.058 ab	19.876 bc	7.751 c				18.791 a	
	<i>P. hyberada</i>	15.041 bc	36.037 a	27.63 ab	12.619 bc				21.244 a	
Interference treatment × Con.	T ₁	19.367b cd	20.38 bc	20.00 bcd	15.283 cd					
	T ₂	19.36 bcd	27.397a bc	27.063a bc	10.25 cd	23.552 a				
	T ₃	19.36 bcd	41.522 a	36.343 ab	9.08 d	27.124 a				
Effect of Con.		20.38 ab	27.956 a	21.549 ab	10.185 b					

* Mean values followed by the same letter are not significantly different according to Duncan's multiple range test (P < 0.05).

Interaction between treatment and concentrations of salicylic acid appears that the higher value for soaking and spray plants at 100 ppm is (41.522) mg/g. From the table, there is numerical differences and there are no other statistically significant values due to the similarity of the letters where the lowest at (15.283) mg/g.

The four concentration of effect of salicylic acid show no significant differences in the among them, although there is numerical differences between them. The highest value was at 100 ppm is (27.956) mg/g , followed by the other concentrations of 200 ppm and 300 ppm, respectively, compared with the control concentration (20.38) mg/g

While there is a significant interaction between plant and salicylic acid treatments found in *P.hybrida* at T₃ treatment (7.751) mg/g, that is the high value while the lower value (11.674) mg/g, the plant *P.niglofera* have high value at treatment T₃ (23.934) mg/g, while the lower value to treatment T₁ at (7.081) mg/g.

There is significant differences in the effect of plants *Petunia* sp. among them, although there is numerical differences between them (21.244 and 18.791) mg/g, respectively.

At the end of the table note that the effect of the treatment of salicylic acid is the largest values (27.124) mg/g while the smaller value (9.378) mg/g, so there is significant differences under the probability level 5%.

Chapter Five

Discussion

5. Discussion

Growth and development of plants, like all organisms, are regulated by various internal, external stimuli. In recent years, SA has been in the focus of intensive research due to its crucial role in the regulation of physiological and biochemical processes during the entire life span of the plants and play key roles in regulating their growth and productivity.

5.1 Effect of SA on Anatomical Axial of *Petunia* sp.

The stomata play an important classification role in the botany kingdom in terms of arrangement and coordination of the subsidiary cells surrounding the guard cells, forming different shapes and patterns on which that are depending by botany scientists in the division of plants to certain levels, in the other hand removal of the deceased into new plant families. As well as the second role, which is no less important physiological aspect and control the process of transpiration and gas exchange, thus leads to the success of the process of photosynthesis or failure, which reflects positively on all metabolic processes intercellular, then the vitality and activity of the plant, This agrees with studies (Pompelli, *et al*; 2010, Amelia, *et al*; 2017).

In terms of the effect of Salicylic acid on stomata, there are two opinions, the first salicylic acid works with ABA together in its negative effect in closing the stomata and lack of leaf area, as well as the speed of death and fall leaf of the plant (Zargham, *et al*; 2014 and Prodhan, *et al*; 2018). The second opinion, which is fully agreed that the Salicylic acid acts as an antagonist or opposite ABA. Thus improving the overall plant characteristics and increasing the flow of Ca^{+2} ion into the guardian's cell and filling with water and then opening the stomata (Alrubae, *et al*; 2012).

When discussing the results of table (4.1) on the leaf area as a reaction to the treatment with salicylic acid, it is found that SA effects are positive within certain levels of SA concentrations, especially at concentrations (100 and 200) ppm. Regarding plant treatments with salicylic acid, first in terms of the effect the third treatment is T_3 (seed soaking + foliar spraying), followed by T_2 (foliar spraying) and finally T_1 (seed soaking).

While increasing the concentration of SA is negative or less influential compared with other concentrations used, this may be attributed to the fact that salicylic acid is a hormone endogenously and when added externally and increase its ratio to critical levels leads to negative effects because it contains a phenolic group increased presence of a cell in which damage occurs, this results in a reduction in the leaf area and this is consistent with what each of the researchers (Nazar, *et al.*, 2015, Jini and Joseph, 2017, Bakundi and Yahaya; 2017). Increase the leafy area due to the salicylic acid to its role in increasing the total content of chlorophyll in the leaves and increasing the relative water content as it stimulates the enzymes responsible for photosynthesis and then accelerating this process leads to the accumulation of processed produces and increase the leafy area (Sadeghipour and Aghaei, 2012).

The number of stomata on the surface of the petunias leaf in the highest value (55200) stomata / leaf at the concentration of 100 ppm to the plant (*P. hyberada*) and the lowest value is at the concentration of 300 ppm at (18960) stomata / leaf is the share of a plant (*P. niglefera*) and this due to the increase of leaf area at the same Concentrations and treatments with salicylic acid and for the same plant see table (4.2) Consistent with the findings of (Bayat *et al.*, 2012 ; Pompelli *et al.*, 2009; Merced and Renzaglia, 2017).

5.2 Effect of SA on the Growth Axial of *Petunia* sp.

Application of salicylic acid to plants increased the shoot growth in different plant *Petunia* sp. That is obviously from table (4.3) results which shows the effects of salicylic acid concentrations in feature shoot length. Therefore the highest value at 100 ppm is (30.80) cm, followed by 200 ppm (28.60) cm in the same treatment T₃ and same plant *P. hyberada* compared with another plant *P. niglefera* was better, on the other hand increasing of length shoot ,perhaps causing to promote SA shoot apex and activation the meristimatic tissue in this area for these reasons the plants are taller more than other plants non treatment with SA they are especially in a lower concentration of salicylic acid , and this explain totally agree with some researchers as (Vicente and Plasencia, 2011; Jazi, *et al.*, 2011;Nazar *et al.*, 2015; Riaz *et al.*, 2017). The explanation for increasing the plant 's height due to the impact of salicylic acid is its function in reducing the concentration of abscisic acid and inhibiting the representation of ethylene that contributed to plant ageing, as well as its function in increasing the output of carbon

representation and photosynthesis and increasing the proportion of hormones such as cytokine and gibberellin (Prabha and kumar Negi, 2014).

Increasing the total soil volume explored by the root of the plant is one of the best ways to improve yield since more water and nutrients may be taken up by the plants. Salicylic acid is effected on root length as show in the table (4.4) showed, noted from results in lower concentration give more positive effects than a higher concentration of SA, in this side of effect the high value (24.30) at 100 ppm in the third treatment, but in the other side found increasing of concentration was inhibition matter as 300 ppm, exactly what happen above (shoot length). Plant depends on the root for the absorption of water and soluble ions as a first job, and the second to fixation of plant, to resistance drought soil and salinity to look and stay plant healthy as said (Jini and Joseph, 2017). Salicylic acid prevents cytokinin and auxin loss in plants and thus increases cell division and plant growth ,The reason for increasing the average of the root length is due to the role of the salicylic acid in increasing the number of leaves and the association of this acid (SA) with amino acids like tryptophan which plays an important role in promoting root growth and formation of AII and its role in increasing the amount of cytokines which play a role in increasing root length and encouraging root growth (Tomaszewski and Thimman 1966; Sheneider and Whitman1974) .

The fresh weights of both stems and roots, respectively, for *Petunia* sp. , shows that there are differences in weights, although not significant, but real numerical differences between plants treated with salicylic acid and as usual tables (4.5) and (4.6) for fresh weights, that the best concentration for SA at 100 ppm, and the values (27.40 ,9.20)g for both fresh weight of shoot and root respectively, surely at T₃ it follows in the same treatment the concentration 200 ppm, while the concentration of SA at 300 ppm, took the lowest values for twice parts of a plant. As for the comparison between the two plants, excellence was in favor of the plant *P. hyberada* at the expense of the plant *P. niglefera*. So this results it was expected, because that increasing biomass of plant and length of roots for both plants and in the all concentration as showed in the tables (4.5) and (4.6) these results are consistent with the findings of Bakundi and Yahaya, (2017) that the effect of different concentrations of salicylic acid increased the fresh weights of leaves plant.

5.3 Effect of SA on the Physiological Axial of *Petunia* sp.

Table (4.9) shows the water content in the cell plant in the all plant body is not for thirsty only but for its medium of metabolic process, on the other hand make plant more rigidity and toughly therefor the effect of salicylic acid and its relation of increasing the water content especially in the concentration 100 ppm from SA and the T₃(seed soaking + foliar spraying) and perfect value is (183.2) %, followed by the effect of 200 ppm in the same treatment and in plant *P.hybrida* , likewise the plant *P.niglofera* took the minimum value at (157.26)%, Roumani *et al.*, 2019 found the increasing of the water content in the cell plant when treated with different levels of salicylic acid ,perhaps causing because accumulation of ions and chemicals compounds lead to increase the osmosis potential to absorption more amounts of water income for plant body, also it is believed that the acid has a role in reducing water loss by transpiration and reducing evaporation by controlling the movement of stomata, increasing the negative osmotic potential of the leaves through the accumulation of amino acids and sugars, which increases the ability and speed of the roots to absorb water and increase the water content of the plant (Naghashzadeh, 2014).

The table (4.10) shows the stability of the membrane ,the plasma membrane playing a crucial role in the life of the cell because it controls the substances entering or leaving the cell. In this point ,SA play improvement of the function of the plasma membrane by increasing the stability of it more protect of the cell., The perfect concentration of SA at 100 ppm and it usually T₃ the value is (68.40)% to plant *P.hybrida* then *P.niglofera* at SA concentration 100 ppm is value (48.40)%.while the lowest value of stability at 300 ppm for *P.niglofera*. The treatment of plants with 300ppm is negative effect compere with control treatment as found by (Johkan *et al.*, 2014) .Salicylic acid facilitated the maintenance membrane functions. This facilitation could be attributed to the induction of antioxidant responses and elevated Ca⁺² uptake that protects the plant from the oxidative damage by salicylic acid (El-Tayeb, 2005; Seneratna *et al.*, 2000).

Table (4.11)and (4.12) show the effect of salicylic acid in prevent permeability of two ions K⁺ and Na⁺ respectively . So using of the SA is positive effects in reducing of measuring of K⁺ and Na⁺ as shows at table (4.10), practically in concentration of SA at 100 ppm for the third treatment and the values were (30.267 and 40.267) for both K⁺

and Na^+ respectively. While increasing of permeability for of K^+ and Na^+ together, whenever in the ascending of SA concentration to be at 300 ppm are values (98.9 and 39.667) these results were damage effects on the passes of the ion across membrane apparently any damage happens for the plasma membrane, surely lead to increasing of permeability of twice ion (K^+ and Na^+) causing for function or job of the plasma membrane cannot keep cell staying healthy without any worst, all this opinion is agreement with what found by (Bijanzadeh, *et al.*, 2019; Samea-Andabjadid *et al.*, 2018) and the second deal with the positive effect of SA in stability of membrane as table (4.10) showed.

Plants as distinguish feature between the organisms in the universe it has ability to manufacture their food, therefore they are called autotrophic organisms, these abilities come from it contains green pigment called chlorophyll. SA application on the *Petunia* sp. in different concentrations as (100, 200 and 300) ppm, then the SA is used in three methods treatment first as seed soaking and the second spraying plants then third collect the both together, in the current study is found all the types of chlorophyll (a, b, a+b and a/b) and carotene they were measured all common in the same concentration was more effect than others, and gave the high values (2.46, 1.083, 3.56, 2.92, 9.63) mg/g, in the T_3 in the same plant *P. hyberada* for the chl. (a, b, a+b, a/b and carotene) respectively as is showed in the tables (4.13), (4.14), (4.15), (4.16), (4.17) but the content plant from chlorophyll and carotene reducing whenever increasing of SA concentrations to reach at 300 ppm these values (0.886, 0.4, 2.46, 1.28, 3.81) mg/g for the above pigments respectively in plant *P. niglofera*.

The table (4.1) for leaf area found the effect of SA increasing this feature, and another table (4.2) is showed impact of salicylic acid in the total numbers of stomata and distribution on the surface of the leaf, perhaps it is once important reason effected in plant content of pigment. While some studies found another reasons as SA is known to affect leaf and chloroplast structure (Uzunova and Popova 2000), stomata closure (Mateo *et al.* 2004; Melotto *et al.* 2006), chlorophyll and carotenoid contents (Chandra and Bhatt 1998; Fariduddin *et al.*, 2003), and the activity of enzymes such as Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and carbonic anhydrase

(Slaymaker *et al.*, 2002; Hayat *et al.*, 2012; Yusuf *et al.*, 2012), and raising the rates of antioxidants in addition to contributing to preventing the destruction of the chlorophyll pigment and increasing the rate of photosynthesis (Yildirim, *et al.*, 2008).

Carbohydrates are the main source of energy in the cell and all the of the organisms, and a plant produces it from the photosynthesis reaction as product materials within that process by converting raw-material to complex compounds in small organelle is called the chloroplast, therefore within relationship with chlorophyll content in a cell specific in chloroplast. Anyway, increasing of carbohydrates is more benefit to cell and for the plant, in the other hand SA from data the table (4.18) shows effecting in the amounts of carbohydrates in the plant *Petunia* sp., particularly at concentration 100 ppm in the both plants (*P. hyberada* and *P. niglofera*) they has values of carbohydrates at (88.33, 88.00) mg/g. Whiles the minimum values at 300 ppm are (50.67 and 44.00) mg/g for both plants. Explaining this results by increasing of chlorophyll in (a and b) in addition carotene content look table (4.13), (4.14), (4.17) shows relationship between the positive relation is meaning that when is increasing of the chlorophyll lead to necessary to increasing of carbohydrates amounts (Azeem *et al.*, 2019). While some studies found another reasons as salicylic acid works to increase the concentration of carbohydrates because it has a role in controlling the internal genes of the plant in stimulating and maintaining organelles and their activity, such as chloroplasts, which leads to the production of carbohydrates, and in the ability of the plant to absorb the elements that lead to an increase in the sugar levels that are used as source for organic carbon (Kaydan *et al.*, 2007).

Mineral nutrients are essential for the growth and development of plants and microorganisms, as they are important factors in the regulation of various physiological and biochemical processes. How each element affects plant's physiological and biochemical processes, (positively or negatively), is unique to each plant. Salicylic acid as phytohormone at level indigenous and exogenous is effecting of the concentrations to anions (positive and negative) charge in the plant, and the ions measured (Na^+ , Ca^{+2} , N^+ , P^{-2} , and K^+) in this study. All the mineral elements are common in one thing are increasing at SA concentration 100 ppm their values (3.833, 10.5, 10.357, 1.060, 5.00) mg/g respectively, in the third treatment (seed soaking + foliar spraying), specially for

plant *P. hyberada* and favor on the *P. niglofera*, then begging reduce of amounts plant *Petunia* sp. from (Na^+ , Ca^{+2} , N^+ , P^{-2} , and K^+) gradually to reach (1.050, 3.1, 1.16, 0.42, 1.66) mg/g respectively. The reason for this result, may be return to effect of SA concentrations of biomass and more efficiency for root system to absorptive these ions from the soil solution, look to table (4.6) and (4.4). The activity of the root system increased by salicylic acid and consequently increase the absorption of the elements by the roots and leaves through the rapid growth and differentiation of the roots and the performance of stomata in addition to its role in regulating the processes of transpiration and respiration and the pulling of the ascending sap, evaporation of water and the accumulation of elements in the leaves and also causes changes in the electrical membranes to encourage absorption of the elements, in addition to the role of the salicylic acid regulatory and stimulating it to produce hormones, or prevent the oxidation of hormones inside the plant, thereby increasing the demand for major elements and their content (Katz, *et al.*, 2002; karlidag, *et al.*, 2009). The second opinion about the increasing of ions or mineral nutrients in the plants are treated with SA as advance step to avoid the drought or salinity in the around environmental, which is the result it matching with (Ahmed, 2009;Manaa *et al.*, 2014).

Protein is very important in the life of the cell, until in the whole organism life protein molecule is possible to make of it hormone or taking another pathway to manufacture enzyme, and may be analysis to produce amino acid in any way protein playing importance role in the cell plant life, therefore any substance is improvement protein be a favorite application on the plant SA effected in increasing protein content as explained from data of table (4.24), particularly at 100 ppm and T_3 and the value was (64.73) mg/g its share for *P. hyberada*, on the other side when increasing of SA concentration lead to reduce protein content is meaning the relationship between a high level of SA concentration and protein content is negative, perhaps return to salicylic acid as internal phytohormone and when added it exogenously reach to toxic levels, the second opinion the same concentration and treatment of SA there happen highest concentration of N^+ in the plant as the table (4.21) showed (Zanganeh, *et al.*, 2019).

Chapter Six

Conclusions & Recommendations

6.Conclusions

1. Using salicylic acid leads to improvement in all properties in the plant *Petunia sp.* and there are some differences between the two plants the best was *P. hybrida* than *P.niglofera*.
2. Lower concentration of SA gives positive effect and rank all most of physiological aspects as water content, chlorophyll type & carotene , carbohydrates, protein, mineral content.
3. A higher concentration of salicylic acid effects more negative on the *Petunia sp.* in the all physiological aspects.
4. Among the methods of treatment T₃(seed soaking + foliar spraying) it is favorite than another methods treatment T₂(foliar spraying) and finally from effecting of SA was (seed soaking).
5. *Petunia sp.* can be used as model in biological studied ,because it has short life and easily effected by any chemical material or environmental condition.

7.Recommendations

1. Application of salicylic acid for its positive effect on studied traits at concentrations below than (100 ppm) in later studies.
2. Use Monocotyledon plant treated with salicylic acid, and recognizing changes in these plants for better or worse.
3. Molecular study of *Petunia sp.* is treated with salicylic acid and recognizing whatever happening of geneing map as response to treating SA.
4. Agriculture of seeds is produced from *Petunia sp.* in the another season and compares it with the first generation.
5. More studies on the flowers of *Petunia sp.* are treated with salicylic acid in variety of different concentration & treatments.
6. Medical uses of *Petunia sp.* because of income from plant family is famous in medicals effects like *Datura* should be make chemical analysis and test biological activity for *Petunia sp.* As antibacterial ,antifungal and antioxidants.

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الملخص

اجريت التجربة في البيت الزجاجي التابع لجامعة المثنى ، كلية التربية للعلوم الصرفة ، قسم علوم الحياة خلال موسم النمو في ديسمبر 2018. والهدف من ذلك هو دراسة تأثير تركيزات مختلفة من حامض الساليسليك وهي (100،0 ، 200 ، 300) جزء في المليون اضيفت بثلاثة طرق مختلفة وهي (نقع البذور ، رش النبات ، نقع البذور ورش النبات) على النمو وبعض الجوانب الفسيولوجية والتشريحية لنوعين من نبات البتونيا وهما *P.niglofera* و *P.hybredia*. تم إجراء التجربة وفقاً للتصميم العشوائي الكامل (C.R.D) بثلاثة مكررات ، وأظهرت النتائج التأثير المعنوي لحامض الساليسليك عند التركيز 100 جزء في المليون للمعاملة (تنقع البذور ورش النبات) وتفق الصنف *P.hybredia* على الصنف النباتي الآخر الذي يسمى *P.niglofera* في صفات النمو الخضري مثل ارتفاع النبات (30.80) سم ، طول الجذر (24.30) سم ، الوزن الرطب للمجموع الخضري والجذري (27.40 ، 9.20) غم ، كذلك الجانب الفسيولوجي مثل الكلوروفيل $a+b$, a/b , b/a والكاروتين بالإضافة إلى الجوانب الأخرى مثل المحتوى المائي النسبي (183.28) % ، ثبات الأغشية (68.40) % ، محتوى المعادن مثل النيتروجين (10.357) ملغم / غم بوتاسيوم (5.00) ملغم / غم من الصوديوم (3.833) ملغم / غم من الكالسيوم (10.5) ملغم / غم من فوسفات (1.060) ملغم / غم ، كربوهيدرات (88.33) ملغم / غم ، بروتين (64.73) ملغم / غم بينما ارتشاح الأيونات K^+ ، Na^+ اظهر الحامض تأثيرات معنوية في تقليل ارتشاح ايونات K^+ و Na^+ (30.267 ، 4.617) وعندما نتحدث عن الجانب التشريحي ، نرى أن حامض الساليسليك كان له تأثير في زيادة مساحة الورقة (15.333) سم² وزيادة عدد الثغور (55200) ، بينما كانت أقل القيم للمعاملة الثالثة تسمى تنقع البذور وللتركيز 300 جزء في المليون للصنف النباتي *P.niglofera* في صفات النمو مثل ارتفاع النبات (13.60) سم ، طول الجذر (15.867) سم ، الوزن الرطب للمجموع الخضري والجذري (16.55 ، 0.36) غم ، والجانب الفسيولوجي مثل الكلوروفيل $a+b$, a/b , b/a و كاروتين بالإضافة إلى الجوانب الأخرى مثل المحتوى المائي النسبي (46.15) % ، ثبات الأغشية (25.50) % ، محتوى المعادن مثل النيتروجين (1.16) ملغم / غم ، البوتاسيوم (1.66) ملغم / غم ، الصوديوم (1.050) ملغم / غم من الكالسيوم (3.1) ملغم / غم ، الفوسفات (0.42) ملغم / غم ، الكربوهيدرات (44.00) ملغم / غم بروتين (8.8) ملغم / غم في حين أن ارتشاح الأيونات مثل أظهر K^+ ، Na^+ أظهر الساليسليك تأثيرات إيجابية في تقليل قياس K^+ و Na^+ (98.9 ، 39.667) وعندما نتحدث عن الجانب التشريحي ، نرى ان لحامض الساليسليك تأثير في زيادة مساحة الورقة (5.00) سم² وزيادة عدد الثغور (18960).

الإقرار

نحن نشهد كأعضاء لجنة مناقشة قد اطلعنا على هذه الرسالة الموسومة ب (تأثير حامض الساليسيليك وطرق اضافته في بعض صفات النمو المورفولوجية والفسولوجية لنوعين من نبات البيتونيا). وقد ناقشنا الطالبة (زهراء حمزة علي) بتاريخ 18 / 5 / 2020. ووجدنا بأن الرسالة تفي بمستوى الحصول على درجة الماجستير في علوم حياة.

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جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة الموصل

كلية العلوم

قسم علوم الحياة

تأثير حامض الساليسيليك وطرق اضافته في بعض صفات النمو المورفولوجية والفسولوجية لنوعين من نبات البيتونيا

رسالة مقدمة كجزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

زهراء حمزة علي

بكالوريوس علوم الحياة ٢٠١٣

بإشراف

أ. د فائق حسن الراضي

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