The Influence of an Addition of the Salicylic Acid (SA) in the Physical and Chemical Characters of the Burley Tobacco Leaves

Tarek Hasan[1], Mahmoud Hasan[2], Ramez Mohammad[3]

1. PhD. Student, Plant Protection Department, Agriculture Faculty, Tishreen university, Lattakia - Syria.

2. Professor, Plant Protection Department, Agriculture Faculty, Tishreen University, Lattakia - Syria.

3. Assistant Professor, Nutritive Science Department, Agriculture Faculty, Tishreen University, Lattakia – Syria

Abstract

The study showed the influence of the Salicylic Acid (SA), which used to protect Burley Tobacco from Black Stem Disease, on the physical and chemical characters of the leaves according to 4 concentrations 0.25, 0.5, 1 and 2 millimoll by two used methods: irrigation and spraying.

Results showed that the treatment by the concentration 0.5 millimoll had given increased the apparent density reached to 47.52% compared by healthy control 26.37%, and chloride percentage 0.61% and 0.72 % to healthy control which considered positive quality to burning character, and good contain of protein 14.1% and 11.43% to healthy control, and low contain of non protein nitrogen (NPN) 2.09%.

Key Words: *Salicylic Acid (SA), Burley Tobacco, apparent density, chloride, protein, non protein nitrogen (NPN).*

I. INTRODUCTION

Tobacco is one of the most important industrial crop at the world [16]. Burley tobacco used in cigarettes industrialize, and entered with 33% in American cigarettes mixture, in addition to Virginia and Oriental tobacco. Burley tobacco is distinguished by its high ability to made cigarette, high produce and good burning [10].

Tobacco black stem disease, which resulted from the fungus *Phytophthora parasitica* var *nicotianae* (B. de Haan), considers one of the most dangerous diseases which damages plants and destroys tobacco in the fields [13].

This disease causes losses by millions dollars to the both of Burley and Flue – Cured tobacco every year in South America [18], [14], as well as annual losses reach to 10 million dollars in North Carolina State [15]. In Georgia State, the black stem disease caused losses ranged between 2-0.1% in the last years [5].

The disease management is achieved by integrating different methods like the agricultural one and the chemical control such as using the Metalaxel deriveds [14] [17] [15] [7]. Recently, the biological control was used as an alternative method instead of industrial chemical fungicide, where the biocontrol has high levels of safety, and less environmental effects. In the present time, there are a new direct side of control, "IAR": the induced acquired resistance in plant which aimed plants to induce natural defense mechanisms against the pathogens. For this aim, many compounds were used and given a good efficacy like Salicylic acid (SA) to induce acquired systemic resistance which had given good results to control black stem disease of tobacco, but we have to know it's influence on the physical and chemical qualities of the leaves.

So this study searched in the changes in the physical and chemical characters of the burley tobacco leaves which treated by SA as irrigation and spraying methods.

II. MATERIAL AND METHODS

A. Salicylic Acid (SA)

Salicylic acid is Known as one of the important phenolic compounds in the plants [9]. It considers a plant hormone which had a major role in arrangement the growth and development of plant [11]. Re. [12] used SA to protect varieties of tobacco in Syria (Burley and Virginia) from Potato Y Virus. His results showed increasing in yield, sugars ratio and NPN ratio, and reducing in protein ratio in all SA treatments comparison to control which infected by Potato Y Virus.

B- Planting Seedlings

This study had done in the Syrian Coast - Tartous at Samerian village. The soil was prepared by adding each of the manure and the mineral fertilizer. The soil had planted by healthy seedlings, 60 days age, 12 cm length, 6 leaves, at 15 /5 /2016. Split-split design was used with 3 replicates, every replicate was 10 plants [12].

C. Burley 21 Variety, Rodesy Tobacco (Br21,R)

This variety is 175-190 cm as an average of the length, 26-30 leaves. The leaf is light green, it's length is 50-60 cm. The panicle is great and branched, it cuts up when 75% of flowers are blooming. It planted in coast and interior land by irrigation methods.

Leaves dried in shadow to become reddish brown. The tissues of the dry leaf are cohesive, elastic, thin, and have a special smell and taste. Nicotine ratio is aboute 1.5- 2 % in case the panicle did not cut up [1].

D. Treatment by Salicylic Acid (SA):

Four concentrations of SA were used in this study, 0.25, 0.5, 1 and 2 millimol /liter (mM/ liter). It used by two methods:

a- irrigation as 30 milliliter/ plant

b- spraying as 10 milliliter/ plant (ml/liter) Each of both methods was applied before 72 hours of infection by Phytopathogenic fungus. The control was prepared by distilled water, 10 ml for spraying method and 30 ml for irrigation method [3].

E. Infection by Phytopathogenic fungus:

The infection by the fungus *Phytophthora parasitica* var *nicotianae* was done after 72 hours of treatment by SA. The infection was performed using a disc (1 cm) of a fungal colony (15 days age) by placed it in a hole inside the root and fill up with earth then irrigated by water [19], [8].

F. Leaves Harvest and Drying

Leaves harvest had made in the physiological ripe period after 40 -50 days. Average of 4-5 leaves from every plant in every harvest process. Harvest leaves dried in shadow according of information of **G**eneral **O**rganization of **T**obacco in Syria (**GOT**). It begins by yellowish stage until change the leaves color (from green to yellow), then dried leaf which became reddish brown color. This stage needs 18-22 days. After this, the main rib dried 15-20 days [12]. Results analyzed by using GenStat- 12 and compared between averages by used **L**east **S**ignificant **D**ifference LSD 1%.

G. Determination An Important Physical Character of Dry Leaf Burley Tobacco [6],[22].

- Apparent Density of Burley Tobacco Leaf.

It's important physical character connected with the structure of tobacco leaf which contain chemical contents. So the tobacco leaf with high apparent density had a dark color, but when it has low apparent density it will has light color. Generally apparent density of leaves increased from basic leaves to upper ones. Apparent density evaluated by taken 10 leaves from each treatment, then take 4 discs from every leaf by used Zomba (mineral tube internal qatar 1.6 cm). In this case the total space of 40 discs 80.38 cm^2 which placed in drier in 105 c° for 1 hour or 95 c° to 3 hours, then calculated by equation.

Apparent Density= Difference×100/ total area spaces

Difference= pot weight after dry – pot weight empty

Total area spaces= $\pi r^2 \times 40$

Whenever the apparent density increased, the leaves become will and have good contents of materials.

H. Determination some chemical contents in leaves burley tobacco.

Tso 1990_c found the percentage of chloride level in burley tobacco leaf between 0.5 -1 % and when it increased of this levels, the burning characters will be bad because formulation of non dissolved chloride salts in burning area [22]. But protein nitrogen when it's percentage increased in burley tobacco leaves considered positive thing. So it increased leaf thickness and spongy texture to absorption melliferous materials during industry stage. Protein compounds considered important, nitrogen compounds in burley tobacco leaf in this case it will be positive to dry leaves quality [6], [20]. Tso 1990_c determine the percentage of nicotine in dry leaf between 2.74-4.18.

All this chemical tests on simples with took from burley tobacco dry leaves in the chemical tests laboratory in General Organization of Tobacco (GOT) in Syria – Lattakia (Jable- Al-Rmaeli) according to tests methods which formed by [4] with some adaptations by A. O [2] by take mixture of leaves in order to doing chemical tests then recorded results and tested statistician by Genstat-12.

H.1- Chloride Determination (by Calibration Method)

200 mg had taken from dry simple (Tambling burley tobacco dry leaf) placed in pot then supply 3 ml $Ca(COOH)_2$ 6% then dried on the flam then placed in oven $500C^\circ$ for 4 hours then supplied hot distilled water to the simple about 15 ml then filtrate the solution in Erlenmeyer flask and washed the funnel and blotting paper few times by hot distilled water until volume of simple become 50 ml. Supply to the simple solution about 2 drops of K_2CrO_4 5% then calibrated by $AgNO_3$ solution normality 0.01 titre until to obtain the red color which still above 5 seconds by shake and determined the chloride Percentage by an equation:

$$Cl\% = \frac{V \times 0.01 \times 35.5 \times 100}{1000 \times simple\ weight}$$

V: Consumption $AgNO_3$ volume (ml)

0.01 Nitrate Normality

35.5 Chlorine equal weight

100 percentage, **simple weight**: after loss humidity (mg)

H. 2- Determination Protein Percentage (Kjeldahl method)

Nitrogen determined by Kjeldahl method so the long protein digested by concentrated sulfuric acid 98% then the nitrogen ammonic acid changes to ammonium sulfate. After digested completed distilling process take place to throw out ammonia from ammonium sulfate by adding sodium hydroxide with heating so ammonia assembly by H_3PO_4 so ammonium borate formatted which calibrated as the final stage by standard HCL with presence of suitable index to determined the calibration end.

Percentage of nitrogen of proteins defined by equation:

$$\mathbf{NP\% = \frac{14 \times y \times v \times 100}{w}}$$

NP%: Nitrogen of Protein Percentage
V= volume ml HCL which used in calibration.
Y= acid calibration = 0.1
14=every milli equivalent of ammonia contain 14 mg nitrogen.
W= dry weight without humidity (mg)
So when proteins contain about one sixth weight nitrogen 16% nitrogen so that we find the **protein coefficient** =100/16=6.25 Thus the protein percentage%= N ×6.25
Note: to determined Non Protein Nitrogen (NPN) the same last steps may applicated except the wished the NPN by CH_3 COOH at the end the determined nitrogen is the total nitrogen so:
NPN %= N(total)% - (N) proteins%

H. 3- Determination of Nicotine Percentage (by Calibration).
Alkaloids of tobacco extracted by used mixture of (Benzene and chloroform) in presence of Barium hydroxide $Ba(OH)_2$ then determined nicotine in the extract by $HClO_4$

$$n\% = \frac{V \times Y \times 162 \times 100}{W}$$

n % = nicotine percentage
so V×Y×162= milli equivalent ×162
results were statistically analyzed by Genstat-12 and compared between averages by account least significant difference LSD 1% between the studying treatments.

III. RESULTS AND DISCUSSION

1-Effect of Salicylic Acid (SA) Addition on The Apparent Density of Burley Tobacco Leaves.

Table (1) showed distinct increase in apparent density on burley tobacco leaves which treated by SA four concentrations. So it was good and positive quality. Pest treatments were by concentration 0.5 mM (millimoll) by irrigation method which gave apparent density 47.52%, followed by concentration 2 mM by spraying method which gave 44.53% apparent density compared by the healthy control 26.37%, and without any significant differences between this treatments and healthy control, this results showed good leaves with good quality and good content of materials.

Table (1:) Effect of SA addition on the apparent density of the burley tobacco leaves

Treatments	Apparent density
Healthy control	26.37^{a}
Infected control	35.95^{c}
SA2 +phyto i	43.16^{de}
SA2+ phyto s	44.53^{ef}
SA1+phyto i	36.07^{c}
SA1+ phyto s	35.82^{c}
SA0.5 +phyto i	47.52^{f}
SA0.5+ phyto s	31.97^{b}
SA0.25 +phyto i	36.07^{c}
SA0.25+ phyto s	39.56^{cd}
LSD 1%	5

Treatments with similar letter indicated to no significant differences between treatments SA2: Salicylic acid with concentration 2 mM . **phyto** : treatment infected by *Phytophthora parasitica var nicotianae*, **i** irrigation method , **s** spray method.

2- Effect of Salicylic acid addition on Nicotine, Chloride, Protein and non Protein nitrogen (NPN) in burley tobacco leaves:

Table (2) showed a clear effected of all SA concentrations in treatments on **nicotine** percentage in burley tobacco leaves. The high percentage was 2.69% when it treated by 0.25 mM by irrigation method followed by 2mM concentration with percentage 2.45% by spray method. It is showing no significant differences between treatments. The limits of nicotine percentage which determined by Tso 1990_e between 2.78- 4.18%.

For **chloride** percentage, table (2) showed the treatment 0.5mM concentration with irrigation method gave less chloride percentage 0.61% , while the spray method for the same concentration 0.5 mM gave 0.62% by noted there are no significant difference between this two treatments, high chloride percentage was by 2mM concentration by irrigation method this concentration gave a negative quality to side the burning burley tobacco leaves because chloride salts formation which non dissolved in burning area according to Tso 1990_e.

Table (2): Effect of SA on some chemecal characters of burley tobacco leaves

Treatments	n%	Cl%	Pr%	NPN%
Healthy control	1.95^{b}	0.72^{c}	11.43^{bc}	1.75^{a}

Infected control	1.06^{a}	0.67^{abc}	15.18^{h}	2.28^{bcde}
SA2 +phyto i	1.32^{a}	0.88^{f}	12.68^{f}	2.23^{bcd}
SA2+ phyto s	2.45^{c}	0.70^{bc}	11.13^{b}	2.79^{f}
SA1+phyto i	2.06^{b}	0.82^{df}	12.28^{e}	2.56^{ef}
SA1+ phyto s	2.1^{b}	0.74^{cd}	9.42^{a}	2.16^{bc}
SA0.5 +phyto i	2.06^{b}	0.61^{a}	14.1^{g}	2.09^{b}
SA0.5+ phyto s	2.53^{c}	0.62^{ab}	11.77^{cd}	2.52^{def}
SA0.25 +phyto i	2.69^{c}	0.69^{abc}	11.47^{bc}	1.79^{a}
SA0.25+ phyto s	1.1^{a}	0.74^{cde}	12.03^{de}	2.43^{cde}
Stander	1.2-1.99	0.5-1	9.38-11.56	0.85-1.09
LSD 1%	0.443	0.114	0.537	0.397

Treatments with similar letter indicated to no significent differences between treatment
.n= nicotine, Cl= chloride, Pr = Protein

The protein percentage increased in infected control reached to 15.18%, followed by SA 0.5 mM by irrigation method 14.1% with significent difference between the last two treatment as noted in table (2). Increased protein percentage positive and desirable quality in burley tobacco leaves. Also the table (2) showed increased in the no protein nitrogen (NPN) percentage reached to 2.79% when used SA 2 mM treatment by spray method, followed by 2.56% by SA 1 mM treatment by irrigation method , and noted no significent difference between it's. Less percentage of (NPN) was in healthy control 1.75% .

IV CONCLUSIONS

Pest treatment of SA was 0.5 mM by irrigation method leaded to burley tobacco leaves with high apparent density 47.52%, and good content of compounds as nicotine 2.06% and high protein percentage 14.1% and low contents of NPN 2.09% , also a low content of chloride percentage 0.61%

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