



## Evaluation of the Efficacy of Salicylic acid and Nicotinic acid to Control Collar Rot and Root Rot Caused by *Sclerotium rolfsii* and Enhance the Productivity of Fenugreek (*Trigonella foenum-graecum* L.) in Egypt



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RECENTLY, fenugreek (*Trigonella foenum-graecum* L.) has been subjected to several attacks by certain soil-borne fungi including *Fusarium moniliforme*, *F. semitectum*, *F. solani*, *Rhizoctonia solani*, and *Sclerotium rolfsii* causing damping-off, collar rot, and root rot which are considered serious diseases leading to delayed growth and subsequent collapse of some infected plants. Five fungal species were isolated from naturally infected fenugreek plants, and *Sclerotium rolfsii* was the most frequently isolated fungus. *Sclerotium rolfsii* was the most virulent among all tested fungi causing the highest percentages of damping-off, collar rot, and/or root rot in the Pathogenicity test. The results confirmed that 10mM salicylic acid (SA) and nicotinic acid (NA) followed by 8mM were more efficacious in inducing resistance against collar rot and root rot diseases, they decreased disease severity and the number of dead plants. These results reflected improving plant growth parameters and yield production during the two growing seasons. Also, SA and NA (10mM) significantly increased the vegetative characteristics plant fresh and dry weight, and yield parameters *i.e.*, average pod weight (g) compared with untreated control. Furthermore, the chemical composition of seed showed a significant increase in total phenol, flavonoids, trigonelline and carbohydrate content. The results suggested that exogenous SA (10mM) application can be used as a potential inducer of systemic acquired resistance, a promising approach for successfully managing collar rot and root rot diseases caused by *Sclerotium rolfsii*. Additionally, SA (10mM) treatment increased quality, quantity of fenugreek yield and can be used as a safe strategy against these diseases.

**Keywords:** Collar rot, Damping-off, Fenugreek, Root rot, Salicylic acid and nicotinic acid, *Sclerotium rolfsii*.

### Introduction

Fenugreek (*Trigonella foenum-graecum*, L.) is an annual plant that belongs to the family Leguminosae, it is known as one of the oldest medicinal plants. It's widely distributed in the Mediterranean basin, India and largely cultivated in Egypt; reached 3567 feddans during 2019/2020 (Ahmad et al., 2016; Anonymous, 2020). Fenugreek has become of ecological, agronomic, and industrial importance, which can

fix atmospheric nitrogen in the soil and reduce the need for nitrogen fertilizers for the plants in crop rotation and as a desert lands crop due to low water requirements. The crop is ranked important among seeds spices to request in the international market, where extraction of the alkaloids and the steroid "diosgenin" from their seeds (Acharya et al., 2014). A lot of recent researches identified many health benefits of fenugreek seed like antimicrobial, antiviral, antitumor, anti-diabetic anti-inflammatory, anti-hyperglycemic,

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hypcholesterolemic effects and antioxidant activity (Kumar et al., 2013). Also, It showed to normalize the blood circulation by making body active and energetic (Sudha & Mathangi, 2013). The medical properties of fenugreek could be due to the bioactive compounds (trigonelline, galactomannans, phenols, flavonoids, carotenoids, coumarins, proteins, saponins and fats). Fenugreek seeds act as a dietary supplement and significantly reduce urine sugar, renal hypertrophy and glomerular filtration rate (Ulbricht et al., 2008). Trigonelline (1-Methylpyridinium-3-carboxylate) is a pyridine alkaloid and a vitamin B3 (nicotinic acid) derivative (Zeiger & Tice, 1997) have many physiological effect such as anti-diabetic, hypolipidemic, anti-cancer activity anti-mutagenic, and neuroprotective (Mohamadi et al., 2018).

Recently, damping-off, foot rot, collar rot, root rot and wilt diseases are destructive soil-borne fungal diseases attacking fenugreek plants at different stages of growing plant, caused by *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Sclerotium rolfsii*, that affect both germination of seeds and seedling death at an early stage, which have become one of the factors constraints to the growers of fenugreek and resulting in severe economic losses in Egypt and worldwide (Ali et al., 2018; Khalequzzaman, 2019; Yadav et al., 2019; Meena et al., 2020; Mohamed & Hagaggi, 2021; Ghule et al., 2021; Kasem et al., 2023).

*Sclerotium rolfsii* Sacc. is a devastating soil-borne plant pathogen that causes a broad range of symptoms that vary according to plant host species, above 500 plant species worldwide, where causing southern blight of groundnut, foot rot in brinjal, collar rot in soybean and fenugreek, and stem rot & pods rot in peanut (Cilliers et al., 2003; Chaurasia et al., 2014; Pawar et al., 2014; Khare et al., 2014; Abo-Zaid et al., 2021). Control of this pathogen is limited or not effective due to sclerotia survive for extended periods in the soil and on plant debris as primary inoculum for the following season (Kumari et al., 2021).

Plant-resistance inducers, *i.e.*, salicylic acid (SA), and nicotinic acid (NA) are gaining importance as substitutes to chemical fungicides and also their ability to stimulate the plant's own resistance mechanism both locally and systemically (SAR) (Walters et al., 2005;

Bawa et al., 2019; Narasimhamurthy et al., 2019). Plant resistance inducers play a vital role in plant protection, which have caused a variety of defensive reactions such as affecting the production of phenolic compounds, the accumulation of secondary metabolites, and activation of various defense-related enzymes in treated-plants depending on the type of chemical elicitors used (Kumar & Bains, 2018; Tripathi et al., 2019; Abdel-Rahman et al., 2021; Asadollahei et al., 2023). Plant-resistance inducers do not have any antibiotic activity, which are biodegradable and safe for use as an alternative for disease management (Benhamou, 1996; Yang et al., 2019; El-Garhy et al., 2020).

Nicotinic acid (pyridine-3-carboxylic acid), also known as vitamin B3, is a water-soluble vitamin, which plays a role in the induction of antibacterial and antioxidant activities and is a well-characterized constituent of the pyridine dinucleotide coenzymes NADH and NADPH, which are involved in many enzymatic oxidations-reduction reactions in living cells (Lourenc et al., 2007; Sadak et al., 2010). Also, it alleviates the effects of many environmental stresses by inducing and regulating secondary metabolic accumulation and/or the manifestation of defense metabolism in plants, as well as increases the crop resistance to diseases in several plant species (Berglund, 1994; Berglund & Ohlsson, 1995; Abdel Aal et al., 2012).

Salicylic acid (SA) is a phenolic compound, which is distributed in many plant species. SA plays a key role in a plant's growth, and development, which is a natural product of phenylpropanoid metabolism and induces resistance responses in plants against pathogens (Cseke et al., 2006; Abdel-Rahman et al., 2021) as well as acts as a growth regulator and flowering of plants. As well as, its effects on the physiological processes related to the growth and development of plants (Vega et al., 2022).

The objective of this work was to evaluate the efficacy of salicylic acid and nicotinic acid at three concentrations (6, 8 and 10mM) via fungicide (Topsin-M) to manage damping-off, collar rot, and root rot caused by *Sclerotium rolfsii* and their impact on growth characteristics, and the quantity, and quality of both yield and seed production of fenugreek plants (cv. Giza 30).

## Materials and Methods

These trials were carried out at Medicinal, Aromatic and Ornamental Plant Disease Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. They were done during 2018/19 and 2019/20 growing seasons. The Legume Research Department, Field Crops Research Inst., ARC, Giza, Egypt, supplied the seeds

### *Isolation, purification, and identification of the associated fungi*

Samples of fenugreek seedlings, the basal portion of the stem at collar region, crowns of plants and root rotted plants showing typical symptoms of the disease were collected from different plantations located at Sharkiya Governorate, for the associated fungi isolation. The infected roots, the basal portion of the stem at collar region as well as crown parts were surface sterilized with 2% sodium hypochlorite solution for 60 seconds and washed serially in sterilized distilled water to remove the traces of the chemical, dried between sterilized filter paper, and then transferred to sterilized Petri plates containing potato dextrose agar (PDA). The Petri plates were incubated at room temperature ( $27\pm 1^\circ\text{C}$ ) and observed periodically for the growth of pure colonies. The developed pure colonies from the plant parts were transferred to PDA slants and incubated at  $27\pm 1^\circ\text{C}$  for 10 days. Single spores or hyphal tips were taken from the developed colonies and transferred, onto (PDA) slants. The fungal isolates were microscopically examined and identified according to their morphological features as described by Nelson et al. (1983) for *Fusarium* species, Domsch et al. (1980) for *Rhizoctonia solani* and *Sclerotium rolfsii*. Such culture tubes were preserved in a refrigerator at  $5^\circ\text{C}$  for further studies. The frequency of the isolated fungi was calculated according to the following formula.

$$\text{Frequency \%} = \frac{\text{No of fungal colonies of each isolated fungus}}{\text{Total number of fungal colonies of all isolated fungi}} \times 100$$

### *Pathogenicity test*

In order to investigate the Pathogenicity of each of the tested isolated fungi, i.e., *Fusarium moniliforme*, *Fusarium semitectum*, *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii*, fenugreek (cv. Giza30) was used. Inocula of

the five tested fungi were prepared by growing each fungus on sterilized sorghum grains and sand medium (100:10 v/v), which was used as a substrate for culture preparation in a conical flask and sterilized. Fresh cultures of seven days old representing the isolated fungi were prepared. Five mm disc was cut from the desired culture to inoculate separately each flask and incubated at  $25\pm 2^\circ\text{C}$  for two weeks for good growth of the fungus tested, during incubation the culture was mixed thoroughly by shaking to get uniform growth of the tested fungus. Clay pots (30cm in diameter) were sterilized by dipping in a 5% formalin solution for 10min and left for two weeks to complete formalin sublimation in open air. Pots were filled with the autoclaved disinfested soil [a mixture of clay soil: sandy (3:1 v/v)]. Soil infestation was achieved by mixing the previously prepared inocula of the desired tested fungus inoculum with the soil at the rate of 2% of soil weight. The infested soil was mixed thoroughly and watered every 2 days for a week before sowing to stimulate the fungal growth and ensure its distribution in the soil. Sterilized un-inoculated sorghum grains and sand medium was added to the disinfested soil at the same rate as control treatment. The experimental pots were arranged as complete randomized block design with six replicated pots for each tested fungal isolate with control (un-inoculated). Apparently healthy fenugreek seeds were surface disinfested by immersing in NaCl (1%) for one min and washed several times with sterilized water and then sown in the infested soil (5 seeds/pot). Meanwhile, seeds sown in pots without inoculum served as control. The seedlings and plants in the pots were observed daily. As soon as, the disease symptoms were evident, disease assessments were performed 15 & 30 days after sowing for pre- & post-emergence damping-off according to Ali et al. (2018), respectively. Meanwhile, the incidence of collar rot and/or root rot of fenugreek was recorded after 45 and 60 days after sowing. The incidence of collar and/or root rot of fenugreek was calculated using the following formula: Incidence of collar rot and/or root rot (%) = number of infected plants/ total number of sown seeds X 100. Then, the pathogen was again reisolated to confirm the infectivity of the isolated pathogen. The pathogenic fungus *Sclerotium rolfsii* was selected as the most virulent to complete this study.

### *Control experiment*

In this experiment; nicotinic acid and

salicylic acid (Sigma Aldrich, USA) were used in this study. While, the fungicide Topsin-M 70% WP (Thiophanate-methyl) was used at the recommended dose (2gm/L water).

#### Experiment layout

This experiment was designed in complete randomized blocks included fifteen treatments, each treatment was replicated three times and each replicate consisted of nine pots (one seed/pot).

#### Pots trial

Clay pots (30cm-diam) were filled with the autoclaved disinfested soil [a mixture of clay soil: sandy (3:1 v/v)]. Soil infestation was achieved by mixing the previously prepared (*Sclerotium rolfsii*) inoculum with the soil at the rate of 2% of soil weight as mentioned under the Pathogenicity test. The pots were irrigated directly and were left under natural conditions of day length and light intensity, and watered regularly to near field capacity with tap water. Fenugreek seeds were soaked in aqueous solutions of each of the tested three concentrations of each treatment, each alone (6, 8, and 10mM) for 45min and planted in the infested soil and un-infested soil as well. On the other hand, seeds were soaked in the fresh suspension of Topsin-M fungicide at (2g/L water) for 90sec and then planted in the infested soil. As well, some of the disinfested fenugreek seeds were left without any treatments and planted in the infested soil with *Sclerotium rolfsii* (positive control) and also, the disinfested fenugreek seeds were left without any treatments and planted in un-infested soil (negative control).

#### Treatments

- 1- Nicotinic acid was applied as a seed treatment at a rate of 6, 8, and 10 mM individually, and the treated seeds were planted in soil infested with *Sclerotium rolfsii*.
- 2- Salicylic acid was applied as a seed treatment at a rate of 6, 8, and 10 mM individually, and the treated seeds were planted in soil infested with *Sclerotium rolfsii*.
- 3- Fungicide (Topsin-M 70%) was applied as a seed treatment at rate of 2gm/L water and seeds were planted in soil infested with *Sclerotium rolfsii*.
- 4- Positive control *i.e.*, un-treated seeds were planted in the infested soil with *Sclerotium rolfsii*.
- 5- Nicotinic acid was applied as seed treatment at a rate of 6, 8, and 10 mM individually, and

the treated seeds were planted in un- infested soil.

- 6- Salicylic acid was applied as a seed treatment at a rate of 6, 8, and 10 mM individually, and the treated seeds were planted in un- infested soil.
- 7- Negative control *i.e.*, un-treated seeds were planted in un-infested soil.

Moreover, twenty-five ml. of each of the tested three concentrations (6, 8 and 10mM) of either salicylic acid or nicotinic acid were watered individually to each pot around the roots of plants thirty days after sowing. Where this rate was enough to cover fenugreek plant roots without surplus (Nada et al., 2014). Plants were watered after one-day post-treatments, to permit maximum fenugreek plant root absorption of each inducer aqueous solution. Then each of the aforementioned treatments was applied four times during the trial, at fifteen days intervals. Pot experiments were carried out during 2018/2019 and repeated in the 2019/2020 growing season.

Chemical fertilizers (NPK) were added as ammonium sulphate (20.6% N), calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) and potassium sulphate (48% K<sub>2</sub>O) at the recommended level in three doses. The 1<sup>st</sup> dose was for all phosphorous amount which was added during soil preparation, the rest (NK) were applied in two equal doses, the 1<sup>st</sup> was applied 45 days after sowing and the 2<sup>nd</sup> was added 30 days from the first dose.

#### Disease assessment

The disease incidence (DI) % was determined by recording pre-emergence, post-emergence damping-off, disease incidence% and % survived plants 15, 30, 60 and 90 days after sowing, respectively according to the following formulas.

$$\text{Pre-emergence (\%)} = \frac{\text{No. of non-emerged seedlings}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{No. of dead emerged seedlings}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of planted seeds}} \times 100$$

Survived plants (%) =  $\frac{\text{No. of survived plants}}{\text{Total No. of planted seeds}} \times 100$

Whereas, disease severity (%) of collar rot and root rot was estimated at the harvest stage (90 days after sowing) based on the progress of yellowing, symptoms of the visible basal portion of the stem at collar region. Root rot, and collar rot percentage of disease severity was determined using the rating scale (0-4 scale) according to Ali et al. (2018) with minor modification where 0 = 0%, 1 = 1-30%, 2 = >30-50%, 3 = >50-70% and 4 = >70-100%.

Disease severity % =  $\frac{[\sum (n \times c)]}{(N \times C)} \times 100$

where: n = Number of infected plants, c = Category number, N = Total number of examined plants and C = The highest category number of infections.

#### *Plant harvest and measurements*

Random samples of fenugreek plants were collected at the harvest stage from each treatment. The following parameters i.e., plant height, number of branches, fresh and dry weights of plant as well as length, number and fresh weight of pods per plant were recorded as well as number and seed yield per plant were determined.

#### *Chemical composition analyses of fenugreek plant and seeds*

##### *Photosynthetic plant pigments*

Determination of photosynthetic pigments (mg/100gm fresh weight), the contents of chlorophyll A, B and carotenoids were determined spectrophotometrically according to Mitic et al. (2013).

Total phenols content was determined in the seed by the method proposed by Amin et al. (2006) as g gallic acid/100g d.w.

Total flavonoids content was measured in seed according to Chang et al. (2002) as g quercetin per 100g d.w.

Quantitative analysis of Trigonelline was determined by High Performance Liquid Chromatography (HPLC) according to the method of Li et al. (2012).

##### *Determination of total carbohydrates*

Total carbohydrates were determined according to the method of Dubois et al. (1956).

The data were expressed as gm/100gm d.w.

#### *Statistical analysis*

The experiment was designed in complete randomized blocks. All recorded data were subjected to the analysis of variance procedures and treatment means were compared using the L.S.D. at  $P \leq 0.05$  of confidence as described by Gomez & Gomez (1984). One-way analysis of variance was done by using the SPSS program version 16.

### **Results and Discussion**

#### *Isolation, Purification and Identification of the Causal Pathogens*

In general, five fungal species were isolated from the diseased fenugreek seedlings and plants collected from different localities in Sharkiya governorate. The isolated fungi were identified as *Fusarium moniliforme* Sheldon, *F. semitectum* Berk & Ravenel, *F. solani* (Mart.) Appel & Wollenw, *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* Sacc. (Table 1). Frequently, *S. rolfsii* showed the highest frequency among those isolated from infected fenugreek either from crown or root samples, being 30 % followed by *R. solani* 23.5 % and *F. solani* 22.5 %. While, *F. moniliforme* and *F. semitectum* were the least isolated ones which recorded the lowest frequencies, being 11 and 13%, respectively. These fungi were previously reported to be associated with fenugreek damping off, foot rot, collar rot, root rot and wilt diseases by other investigators (Rani & Hagde, 2017; Ali et al., 2018; Ramteke et al., 2019; Yadav et al., 2019; Meena et al., 2020; Kumari & Poonia, 2021).

#### *Pathogenicity test*

The results presented in (Table 2) indicate that all the tested fungal isolates were able to infect fenugreek plants and showed variation in their ability to cause damping-off, collar and root rot symptoms in plants compared with control plants under artificial inoculation conditions. It is clear that, the tested isolates were able to cause pre- and post-emergence damping-off to fenugreek seedlings with differences among them. The results showed that emerged seedlings were very susceptible and may die quickly due to infection at pre- and/or post-emergence stage. *S. rolfsii* gave the highest pre- and post-emergence damping-off values, being 26.66 and 20.00%, respectively followed by *R. solani*, being 20.00 and 13.33%,



respectively. Meanwhile, *F. moniliforme* and *F. semitectum* showed the lowest percentages of pre- and post-emergence damping-off, being (13.33-10.00%), respectively. On the other hand, the obtained data showed a moderate variation in the percentages of infection at 45 and 60 days after sowing in the infested soil depending on the causal pathogen. The symptoms of collar rot and root rot recorded during the Pathogenicity test were almost similar to the natural symptoms. Initially, either the collar or root rot infected plants showed gradual yellowing of leaves, drooping and drying for leaves within 45 days after sowing. Eventually, the infected plant lacks lateral root system, stunted growth of the plant, wilted and fungi spread more rapidly and destroyed the root system leading to complete collapse of some plants within 60 days after sowing. The occurrence of disease incidence % was recorded in vary ranging from 13.33 to 26.66%, respectively after 45 and 60 days from sowing. At the same time, plants under *S. rolfsii* stress showed more amount of the disease where they recorded a degree of high infection (26.66%) followed by *F. moniliforme*, *F. solani*, and *R. solani* (20.00%), while *F. semitectum* exhibited the lowest disease incidence (13.33%) after 45-days from sowing, often these infected plants collapsed over time. Data in Table 2 show that *S. rolfsii* was a highly virulent one based on damping-off (% pre- & post-emergence) and collar rot and/or root rot incidence on fenugreek (26.66%, 20.00%, and 26.66%), respectively. Accordingly, it was selected for the further studies based on their pathogenic ability. In fact, the results indicated that *S. rolfsii* is considered one of the important serious pathogens, particularly in the seed germination stage causing pre and post-emergence damping-off, as well as yellowing of leaves and collar rot and/or root rot symptoms

developed at an early stage of plant growth and maybe persisted up to the end of the crop but to a lesser degree. *S. rolfsii* can survive within a wide range of environmental conditions (Pinheiro et al., 2010; Pawar et al., 2014; Dwivdi & Prasad, 2016). Furthermore, significant positive correlation was found between the amount of oxalic acid production by *S. rolfsii* and the virulence of this pathogen of hyphal invasion and infection, as well as their ability to secretion other degradation enzymes causing disintegration of cell walls and killing host tissue (Haveri, 2017; Chen et al., 2020). Noticeably, the preliminary symptoms of infected seedlings were pale brown gradually girdled by lesions, advanced approximately up 1.5 to 2.5 cm above the soil level appeared at the collar region within 30 days from sowing, which have eventually become dark brown lesion, may be after 45 days of infection (Fig. 1). In some cases superficial frosty fluffy mycelial growth of the fungus was observed on the basal portion of the stem near soil surface at collar region, which is one of the characteristic symptoms caused by the fungus. This lesion develops rapidly to girdle basal stem tissues (crown) within 60 days of infection. Hence, plants often decline and may die as a result of infection (Khare et al., 2014; Kator et al., 2015). Similarly, our results are in line with those obtained by Singh et al. (2014), Ali et al. (2018), Khalequzzaman (2019), Kumari & Poonia (2021), Mohamed & Hagaggi (2021), who reported that *F. solani*, *F. semitectum*, *F. moniliforme*, *S. rolfsii* and *R. solani* are able to cause damping-off, foot rot, collar rot and root-rot diseases of fenugreek which reduce the yield of the plant under greenhouse and field conditions. Fungal isolates were re-isolated from the infected plants but not from the control plants to confirm Koch's postulates.

**TABLE 1. Occurrence and frequency (%) of fungi isolated from fenugreek diseased seedlings and plants, collected from Sharkiya governorate**

Fungi	No. of isolates	Frequency (%)
<i>Fusarium moniliforme</i>	22	11.0
<i>Fusarium semitectum</i>	26	13.0
<i>Fusarium solani</i>	45	22.5
<i>Rhizoctonia solani</i>	47	23.5
<i>Sclerotium rolfsii</i>	60	30.0
Total	200	100
L.S.D.at 5%:	--	0.77

TABLE.2. Pathogenicity test of the fungi isolated from fenugreek plants 15, 30, 45 and 60 days after sowing in artificially infested soil with each of the tested fungi

Fungi	Disease assessment			
	% Damping-off		% Root rot and /or collar rot incidence after (days)	
	Pre-emergence after (15days)	Post-emergence after (30days)	45 days	60 days
<i>Fusarium moniliforme</i>	13.33	10.00	20.00	26.66
<i>Fusarium semitectum</i>	13.33	10.00	13.33	13.33
<i>Fusarium solani</i>	20.00	10.00	20.00	26.66
<i>Rhizoctonia solani</i>	20.00	13.33	20.00	26.66
<i>Sclerotium rolfsii</i>	26.66	20.00	26.66	26.66
Control	00.00	00.00	00.00	00.00
L.S.D.at 5%:	0.69	0.38	0.79	0.28

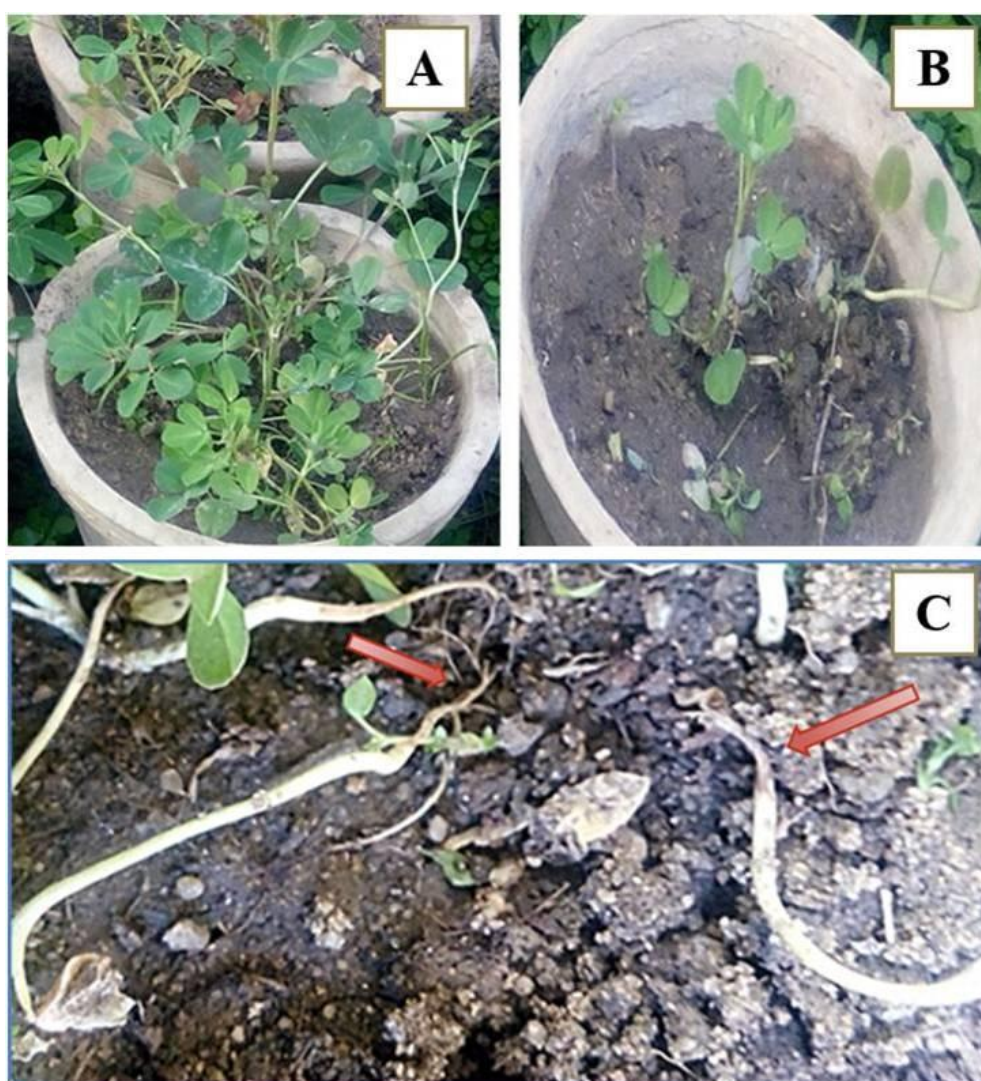


Fig. 1. Healthy fenugreek plants (A). The first symptoms appeared; as reduced pre- and post-emerging seedlings, and emerged seedlings were very susceptible (B). Symptoms of collar rot of fenugreek incited by *Sclerotium rolfsii*, appeared as pale brown to dark brown lesions encircling the stem near the soil surface (C). Infected fenugreek plants grown in soil artificially infested with *Sclerotium rolfsii* (B & C)

### Control experiment

*Effect of salicylic acid (SA) and nicotinic acid (NA) on damping-off, collar rot, and root rot incidence as well as survived plants of fenugreek plants grown in soil artificially infested with S. rolfsii*

The systemic acquired resistance (SAR) has attracted interest as a strategy for controlling plant diseases. The resistance was apparent as a reduction in disease incidence compared with positive control. As shown in Tables 3 & 4, the two organic acids at the three tested concentrations (6, 8, and 10mM) and the fungicide Topsin-M 70% (2gm/L) significantly reduced percentages of pre- and post-emergence damping-off, collar and/or root rot disease incidence and disease severity, as well as increased percentage of healthy survived plants of fenugreek (cv. Giza 30) compared to the untreated control during the two growing seasons under artificially soil infestation with *S. rolfsii* conditions. In general, the results illustrated that the effectiveness of the two tested organic acids varied according to the tested acid and their tested concentrations. In the first growing season, nicotinic acid at 10mM was the superior treatment for minimizing pre-emergence damping-off (3.7%) followed by salicylic acid at 10mM and nicotinic acid at 8mM (7.41%), while salicylic acid at 8mM and Topsin-M showed moderate effect in this respect (11.11%) when compared to the control (25.92%). The same trend was observed approximately in the second growing season (2019/20). On the other hand, salicylic acid at 10 mM occupied the first rank in reducing post-emergence damping-off, recorded 3.70% as well as 1.85% disease severity of collar rot and/or root rot and increased the survived plants to 81.48%, 77.78%, respectively during the two growing seasons. Salicylic acid at 8mM and Topsin-M were the most efficient in this regard which recorded the lowest post-emergence damping-off, being (7.41%) and significantly reduced the disease severity, being 2.78 and 4.63%, respectively as well as, significantly increased the survived plants, being 74.07 and 62.96%, respectively followed by nicotinic acid at 10mM, being (11.11, 3.70, and 74.08%), respectively compared to the control (18.52, 18.52 and 18.53%), respectively in the first growing season (2018/19). It was noticed that the second season mostly had the same pattern as the first season with few exceptions. Abdel Aal et al. (2012) indicated that the SA-dependent defense pathway can be activated by the treatment of plants with chemical inducers such as nicotinic acid (NA),

which induces resistance in sesame plants against charcoal rot disease (*Macrophomina phaseolina*). On the other hand, it was clear that 8mM or 10mM concentrations of salicylic acid gave a sharp decrease in the disease incidence (7.41%) compared with untreated control (37.03%) in the first season; in regard, the disease incidence% was higher in the second season than in the first one. Generally, the results showed that these treatments improved the surviving plants by reducing collar rot and root rot disease incidence. The obtained results are in agreement with other researchers who indicated that treatment of the plants with elicitor (SA) can supply a significant degree of protection against pathogens (Sarhan et al., 2018; Tripathi et al., 2019; Yang et al., 2019) as well as delaying the progression of the disease. Evidently, salicylic acid at 10mM was more effective in inducing resistance in plants against damping-off, collar rot and root rot diseases at all different stages of growth for fenugreek plants compared to other tested concentrations of treatments. In this work, our results documented those soaking seeds plus soil treatment (as soil drench) enhanced the protective effect of SA against *S. rolfsii*. Accordingly, the authors suggest using SA alone or combined with chemical fungicides in the field for effective management of collar rot and/or root rot of fenugreek plants. Several previous studies showed that SA can directly impede fungal growth *in vitro* as mentioned by Saloua et al. (2018), Kumar & Bains (2018) and Yang et al. (2019) who found that SA exhibited fungi toxicity toward some soil-borne phytopathogenic fungi; inhibited radial growth, and spore formation of *Fusarium* spp. and *Magnaporthe oryzae* but at different degrees depending on concentrations used and fungal pathogens tested. On the other hand, Ramteke (2019) reported that SA did not possess direct antifungal activity at lower concentrations, but high concentrations of SA reduced sporulation of *F. solani* the cause of root rot of fenugreek. It was clear that SA acts as a toxic chemical to some phytopathogenic fungi. This strengthens the hypothesis that SA activates the signal transduction pathway, thus leading to the expression of systemic acquired resistance, rather than inhibiting the fungus directly (Mettraux et al., 2002). In this respect, Jendoubi et al. (2015) stated that the exogenous application of salicylic acid at 200µM is safe for tomato plants, stimulates plant defense mechanisms against *Fusarium* wilt, salicylic acid treatment induced the accumulation of both defense chemicals (soluble phenolic



compounds), PR1 and a high level of PR-proteins, {glucanase (PR4) and chitinase (PR2)} in leaves of hydroponic tomato plants post-treatment by root feeding. Additionally, they found a gradual increase in peroxidase activity (PR9) and phenyl-amenia-lyase (PAL) in roots and leaves after SA roots feeding. External application of SA played an important role in the highest reduction of charcoal rot of sesame plants, root rot incidence, and disease severity, in addition to maximizing healthy plants and improving all growth parameters tested and yield production under greenhouse and field conditions (Abdel Aal et al., 2012). A similar response was noticed in tomato plants treated with SA under *Rhizoctonia solani* and *Sclerotium rolfsii* stress (El-Mohamedy et al., 2014). Recently, Sarhan et al. (2018) mentioned that the application of SA was among the most efficient chemicals that reduced the disease incidence and severity of white mold disease (*Sclerotinia sclerotiorum*) of snap bean plants, increasing the activities of the antioxidant enzymes peroxidase (PO), polyphenoloxidase (PPO), improving the phenolic contents, and increasing the marketable yield. In this regard, Bektas & Eulgem (2015) and Tripathi et al.

(2019) found that SA is among the most common chemical elicitors known to invigorate many defense-related enzymes and to produce phenolic compounds in plants. Further, salicylic acid is recognized as an endogenous signal, mediating in plant defense, against pathogens leading to systemic acquired resistance (SAR). As well as, plays a vital role in the resistance of pathogens by inducing the production of pathogenesis-related (PR) proteins Métraux (2001). Furthermore, Abdel-Rahman et al. (2021) proved that SA had a higher efficiency in inhibiting the growth of *A. alternata* (*in vitro*), a reduction of black mold severity, and increasing the expression of PR-proteins (PR-1 proteins and  $\beta$ -1,3- glucanase) and defense enzyme (peroxidase) in sweet pepper plants. Exogenous application of SA is often applied for plant disease control as a powerful elicitor, antimicrobial and antifungal, such as for bacterial diseases Narasimhamurthy et al. (2019) and fungal diseases Bawa et al. (2019). Recently, studies on the potential of SA as a fungicides alternative had increased most for its non-toxicity effect on plants and humans (Tripathi et al., 2019; El-Garhy et al., 2020).

**TABLE 3.** Effect of SA and NA at three concentrations on damping-off, collar and root rot incidence as well as survived plants of fenugreek plants grown in soil artificially infested with *S. rolfsii* 15, 30, 60 and 90 days after sowing, respectively during 2018- 2019 growing season

Treatments	*Con.	Disease assessment				
		% Damping-off		% Root rot and/or collar rot incidence (60 days)	% Disease severity of root rot and collar rot (90 days)	% Apparently healthy survived plants (90 days)
		Pre-emergence (15 days)	Post-emergence (30 days)			
Nicotinic acid(NA)	6mM	14.81	18.52	18.52	5.55	48.15
	8mM	7.41	14.81	14.81	3.70	62.97
	10mM	3.70	11.11	11.11	3.70	74.08
Salicylic acid (SA)	6mM	11.11	14.81	11.11	4.63	62.97
	8mM	11.11	7.41	7.41	2.78	74.07
	10mM	7.41	3.70	7.41	1.85	81.48
Fungicide(Topsin-M)	2gm/L	11.11	7.41	18.52	4.63	62.96
*Positive control		25.92	18.52	37.03	18.52	18.53
**Negative control		0.0	0.0	0.0	0.0	100.00
L.S.D. at5%:		4.13	4.01	0.25	0.25	2.34

\* Positive control (untreated plants grown in infested soil), \*\*Negative control (untreated plants grown in un-infested soil), \*Con. (concentrations)

**TABLE 4.** Effect of SA and NA at three concentrations on damping-off, collar and root rot incidence as well as survived plants of fenugreek plants grown in soil artificially infested with *S. rolfisii* 15, 30, 60 and 90 days after sowing, respectively during 2019-2020 growing season

Treatments	*Con.	Disease assessment				
		% Damping-off		% Root rot and /or collar rot incidence (60 days)	% Disease severity of root rot and collar rot (90 days)	% Apparently healthy survived plants (90 days)
		Pre-emergence (15 days)	Post-emergence (30 days)			
Nicotinic acid(NA)	6mM	14.81	18.52	18.52	6.48	48.15
	8mM	11.11	14.81	14.81	3.70	59.27
	10mM	7.41	7.41	14.81	3.70	70.37
Salicylic acid (SA)	6mM	11.11	14.81	14.81	3.70	59.27
	8mM	11.11	11.11	11.11	2.78	66.67
	10mM	7.41	3.70	11.11	1.85	77.78
Fungicide (Topsin-M)	2gm/L	14.81	11.11	11.11	5.55	62.97
*Positive control		22.22	22.22	33.33	26.85	22.22
**Negative control		0.0	0.0	0.0	0.0	100.00
L.S.D. at5%:		4.85	3.06	0.78	0.25	0.38

\* Positive control (untreated plants grown in infested soil). \*\*Negative control (untreated plants grown in un-infested soil), \*Con. (concentrations)

*Effect of salicylic acid (SA) and nicotinic acid(NA) on some plant growth parameters of fenugreek plants grown in soil artificially infested/or un-infested by S. rolfisii.*

*Plant height (cm), fresh and dry weight /plant (gm):* Results in Table 5 indicate that nicotinic acid and salicylic acid (6, 8 and 10mM) caused significant increases in plant height (cm), fresh and dry weights of plant (gm) compared to positive and negative control in the two growing seasons. These increases were at the maximum range due to treatments with nicotinic acid and salicylic acid at 10mM. The recorded values were 37.75, 40.60 and 39.12, 40.11 and 55.85, 55.00 and 59.67, 57.00 for plant height (cm), respectively in both seasons. A similar effect of NA and SA at 10mM was obtained for both fresh and dry weights of the plant (gm). Meanwhile, there was no significant differences between plants treated with fungicide (Topsin-M) and those treated with SA and NA (8 and 10mM) grown in infested soil. Application of SA and NA alleviated the effects of *S. rolfisii* infection. The obtained results are in agreement with those obtained by Mohammad et al. (2019) who reported that foliar spray with SA on fenugreek plant increased fresh and dry weights. Morris et al. (2000) found that the increment in plant height, fresh and dry weights parallel with increasing of SA dose, may be due to the role of SA in regulating transduction

signal and regulation signal of gene expression during cell growth. Another explanation by Rosalein (1992) that SA effect on active compounds induction for the cell speed up the metabolism. Hayat et al. (2007) reported that SA may effect on phenol compounds that are wide spread in plants contributes to the regulation of physiological processes. The Synergism effect of phenolic compounds with other compounds like growth regulator IAA plays an important role in the increment of the volume of cells and cell division. SA has an important role in increment of auxins and cytokinins content (Shakirova et al., 2003).

Hayat et al. (2005) reported that SA has an important role in catalyzing enzymes responsible for anabolism that could be explained the increase in fresh and dry weights affected by growth regulator as well SA acts in dividing cells which leads to more fresh and dry weights. Foda (1987), Deyab (1989) and Mohamed et al. (1989) showed that nicotinic acid sprays on plant at (100mg/L) concentration was the best concentration for increasing the plant height. Noctor et al. (2006) mentioned that nicotinic acid is important component for most physiological processes in plant, and responsible for several biochemical reactions.

**TABLE 5.** Effect of SA and NA on plant height (cm), fresh and dry weight/ plant (gm) of fenugreek plants grown in artificially infested/or un-infested soil by *S .rolfsii* during 2018/2019 and 2019/2020 growing seasons

Treatments		Plant height (cm)		Fresh weight/ plant(gm)		Dry weight / plant (gm)	
		1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Plants grown in infested soil							
Nicotinic acid (NA)	6mM	30.81	32.41	21.09	20.95	6.15	5.11
	8mM	34.94	36.06	23.99	23.51	7.14	6.98
	10mM	37.75	39.12	27.10	26.05	8.39	7.80
Salicylic acid (SA)	6mM	32.67	34.68	20.82	20.92	5.97	6.46
	8mM	36.01	36.36	24.24	22.72	6.82	7.15
	10mM	40.60	40.11	27.08	27.74	7.97	7.77
Topsin-M	2gm/L	35.31	36.43	20.94	21.15	7.03	6.78
*Positive control		28.12	25.44	10.50	10.85	4.08	4.56
Plants grown in un-infested soil							
Nicotinic acid (NA)	6mM	48.33	50.67	30.82	31.00	8.97	7.46
	8mM	51.50	55.24	33.46	33.15	9.81	8.42
	10mM	55.85	59.67	37.78	38.28	11.07	9.97
Salicylic acid (SA)	6mM	48.00	49.19	29.36	31.86	8.47	7.82
	8mM	51.67	52.33	32.51	31.23	9.11	9.31
	10mM	55.00	57.00	37.85	38.62	11.01	10.85
**Negative control		44.97	46.18	27.28	27.01	6.87	7.21
L.S.D. at5%:		2.03	2.50	1.60	2.64	0.97	0.98

\* Positive control (untreated plants grown in infested soil). \*\*Negative control (untreated plants grown in un-infested soil)

*Number of branches, length of pods and number of pods/plant:* Data in Table 6 show that the application of nicotinic acid and salicylic acid at 10 mM had a significant increment in the number of branches, length of pods and number of pods/plant compared to positive and negative controls in both growing seasons. The lowest values were observed in positive control in the two seasons. Meanwhile, there was no significant difference between fungicide (Topsin-M) treatment and salicylic acid and nicotinic acid at 10 mM under infestation soil conditions. These results are in good agreement with those found by Abdul-Hafeez, (2019) who found that foliar application of salicylic acid on fenugreek seed caused significant increment in seed weight/plant and seed yield/fed-dans. Mohammad et al. (2019) recorded that the foliar spray of SA on fenugreek caused an increment in the number of seeds per pod and number of seeds per plant.

Abdi (2020) confirmed that the foliar application of salicylic acid on fenugreek under water deficit conditions caused significant increment in number of pods per plant and weight of 1000-seed. Also, the obtained results are conformed by Bakhom et al. (2019), Dawood

et al. (2019) and El-Bassiouny et al. (2020) on wheat plants and soybean, and Mohamed et al. (2020) who found that the foliar applications with nicotinamide caused significant increment in plant height (cm), number of branches and number of leaves/plant compared with untreated plants.

*Number of seeds, fresh weight of pods and seeds/ plant (gm):* Results in Table 7 show that the number of seeds, fresh weight of pods(gm) and dry weight of seeds (gm)/plant of fenugreek plant were significantly affected by using NA and SA at 8 and 10mM compared to positive and negative controls in both growing seasons. The best results obtained from plants treated with NA and SA at 10mM. The same trend of results was obtained by Abdul-Hafeez, (2019) who recorded that salicylic acid increased pods number, seed weight per plant, seed yield per feddan, weight of 1000-seed and weight of plant. Also, Bakhom et al. (2019); Dawood et al. (2019); El-Bassiouny et al. (2020) confirmed the results on plants of wheat and soybean. Abdi, (2020) showed that salicylic acid has positive effect on number of pods per plant and weight of thousand seed. This could be due to increment in pod number per plant and seed number /pod.

**TABLE 6. Effect of SA and NA on no. of branches, length of pods and No of pods/plant of fenugreek plants grown in artificially infested/or un-infested soil by *S. rolfii* during 2018/2019 and 2019/2020 growingseasons**

Treatments		No of branches/plant		Length of pods/plant		No of pods/plant	
		1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Plants grown in infested soil							
Nicotinic acid (NA)	6mM	3.47	3.60	7.07	8.50	9.67	8.00
	8mM	4.67	5.33	7.64	8.76	12.67	10.00
	10mM	4.97	5.67	8.99	9.51	13.00	10.67
Salicylic acid (SA)	6mM	3.33	4.00	6.92	8.72	10.00	7.00
	8mM	3.67	4.00	9.28	9.42	10.33	8.97
	10mM	4.00	4.67	9.87	9.54	13.00	9.82
Topsin-M	2gm/L	4.33	4.67	9.30	9.27	12.00	9.33
*Positivecontrol		2.67	2.00	6.37	5.80	7.33	5.33
Plants grown in un-infested soil							
Nicotinic acid (NA)	6mM	5.33	5.67	10.12	12.02	15.67	15.50
	8mM	6.67	6.67	11.70	13.13	16.67	18.23
	10mM	7.00	7.33	13.52	13.70	18.00	19.38
Salicylic acid (SA)	6mM	4.67	6.00	10.33	11.09	14.00	14.00
	8mM	6.00	7.00	11.45	11.92	17.67	18.86
	10mM	6.67	7.67	13.89	13.74	17.67	19.54
**Negative control		4.67	4.00	8.70	10.37	13.00	12.33
L.S.D. at5%:		1.13	1.21	1.02	1.15	2.98	1.96

\* Positive control (untreated plants grown in infested soil). \*\*Negative control (untreated plants grown in un-infested soil)

**TABLE 7. Effect of SA and NA on No. of seeds/plant, fresh weight of pods and dry weight of seeds/plant(gm) of fenugreek plants grown in artificially infested/or un-infested soil by *S. rolfii* during 2018/2019 and 2019/2020 growingseasons**

Treatments		No of seeds/plant		Fresh weight of pods/ plant (gm)		dry weight of seeds/plant (gm)	
		1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Plants grown in infested soil							
Nicotinic acid (NA)	6mM	33.33	35.00	6.04	5.35	0.788	0.703
	8mM	40.81	44.33	7.17	6.92	1.463	1.20
	10mM	44.79	47.33	8.34	8.25	1.656	1.84
Salicylic acid (SA)	6mM	49.33	51.67	6.33	6.74	0.814	0.63
	8mM	54.67	73.71	7.49	8.12	1.115	1.09
	10mM	72.00	77.04	7.91	8.65	1.804	1.85
Topsin-M	2gm/L	33.33	34.67	6.96	7.69	1.33	1.19
*Positive control		25.67	17.33	3.07	3.43	0.50	0.55
Plants grown in un-infested soil							
Nicotinic acid (NA)	6mM	52.00	52.00	11.34	10.45	1.53	1.32
	8mM	84.46	71.67	13.47	12.52	2.01	2.06
	10mM	95.67	92.33	14.31	15.00	2.88	2.89
Salicylic acid (SA)	6mM	66.00	73.33	11.49	11.45	1.39	1.27
	8mM	74.40	85.00	13.06	13.34	1.86	1.71
	10mM	89.67	96.00	14.18	14.17	2.74	2.57
**Negative control		46.71	40.67	9.76	10.08	2.65	1.32
L.S.D. at5%:		8.49	7.57	1.11	0.81	0.98	0.49

\* Positive control (untreated plants grown in infested soil), \*\*Negative control (untreated plants grown in un-infested soil).



*Effect of salicylic acid (SA) and nicotinic acid (NA) on the chemical composition of fenugreek plants grown in artificially infested/or un-infested soil by S. rolfii*

*Chlorophyll A, chlorophyll B and carotenoids:* Data in Table 8 show that high concentrations of salicylic acid and nicotinic acid (8 and 10mM) increased chlorophyll A, chlorophyll B and carotenoids compared to positive control in the two growing seasons. The best results were obtained by using nicotinic acid and salicylic acid (10mM). While, NA and SA-treated plants showed decreasing in the chlorophyll A, chlorophyll B, and carotenoids content under *S. rolfii* stress.

The changes in chlorophyll content are used as an indicator of abiotic stress linked to visual symptoms of plant illness and photosynthetic efficiency. Pour et al. (2013) and Selem et al. (2018) showed that salicylic acid treatment of fenugreek under levels of salinity stress caused increment in chlorophyll a, chlorophyll b and carotenoids contents in comparison with control plants. Meanwhile, Abdul-Hafeez (2019) demonstrated that foliar application of salicylic acid increased the content of chlorophylls (A and B) and carotenoids. The positive effect of SA might be due to inhibition of production and accumulation of free radical in plants, which has negative effect. The role of nicotinamide is confirmed by Sadak et al. (2010), Abdelhamid et al. (2013) and Dawood et al. (2019) on sunflower and fababean. On the other hand, Mohamed et al. (2020) showed that the foliar application of nicotinamide caused significant increment in chlorophylls (A, B) and total pigments compared with untreated plants.

*Total phenols, Total flavonoids, Trigonelline and total carbohydrate:* Results shown in Table 9 indicate that NA and SA at concentrations (6, 8 and 10mM) increase total phenols, total flavonoids and trigonelline in fenugreek plant comparing to positive and negative controls. Treatment at (10mM) NA and SA had an enhancing effect on the measured parameters. The phenolic compounds are a group of secondary metabolites that have different biological effects. The most important role is their antioxidant activity in plants (Metwally et al., 2019; Zlatić et al., 2019). Further, they increase the protective effect, and reducing the level of ROS under stress conditions, and facilitating rapid elimination so

that metabolism remains stable.

Nicotinic acid is a precursor in the biosynthesis of trigonelline in fenugreek that may explained the increment in trigonelline in NA supplemented tissue cultures *Moringa olifera* (Mathur & Kamal, 2012).

Also, Mohamed et al. (2015) reported that combined treatment, pre-soaking plus spraying nicotinic acid caused an increment in secondary metabolites in fenugreek seeds such as trigonelline, total phenols and total flavonoids. As well as, Dawood et al. (2019) confirmed the results in faba bean plants. Moreover, Mohamed et al. (2020) showed that treatment with nicotinamide caused significant increment in total phenolic compound. Carbohydrates like sugars (fructose, glucose, fructans, and trehalose) and starch are involved in the response to several stresses, and act as nutrient and metabolite signaling molecules (Couée et al., 2006; Abdel Latef et al., 2017). Moreover, the high accumulation of carbohydrate helps to prevent oxidative losses by scavenging ROS and maintaining protein structure during salt stress. Additionally, the marked increase in carbohydrate levels in fenugreek leaves following SA application corroborated the findings of (El-khodary, 2004; Fahad & Bano, 2012; Jini & Joseph, 2017). Meanwhile, SA application deranges the enzymatic reaction of polysaccharide hydrolysis, which increases the hydrolysis of insoluble sugars and creates an osmotic source that is important to osmotic regulation. At the same time, our results (Table 9) indicate that there is an increase in total carbohydrate content in fenugreek plants. When the concentrations of SA and NA increased from 6 to 10mM, total carbohydrates increases compared with positive and negative controls. The differences between the high concentrations of resistance inducers (10mM) and fungicide (Topsin-M) were not significant in both seasons. The obtained results are in conformity with the results obtained by Mohamed et al. (2015) who reported that nicotinic acid at increased the content of total carbohydrates. Recent studies showed that nicotinamide treatment caused significant increment in total carbohydrates (Mohamed et al., 2020). It was observed that the positive effect of nicotinic acid may be due to its acting as a coenzyme in the enzymatic reactions of carbohydrates, protein and fats metabolism and is involved in photosynthesis and respiration (Robinson, 1973).

**TABLE 8.** Effect of SA and NA at the three concentrations on chlorophyll A, B and carotenoids of fenugreek plants grown in artificially infested/or un-infested soil by *S. rolfii* during 2018/2019 and 2019/2020 growing seasons

Treatments		Chlorophyll A (mg/g)		Chlorophyll B (mg/g)		Carotenoids (mg/g)	
		1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Plants grown in infested soil							
Nicotinic acid (NA)	6mM	0.986	.8480	0.291	0.285	0.428	0.417
	8mM	1.160	1.053	0.315	0.329	0.549	0.3970
	10mM	1.220	1.038	0.318	0.314	0.590	0.433
Salicylic acid (SA)	6mM	0.887	0.816	0.289	0.309	0.430	0.387
	8mM	1.099	0.932	0.313	0.337	0.492	0.430
	10mM	1.270	1.24	0.342	0.349	0.525	0.497
Topsin-M	2gm/L	1.083	0.857	0.317	0.323	0.489	0.437
* Positive control		0.751	0.704	0.261	0.235	0.391	0.375
Plants grown in un-infested soil							
Nicotinic acid (NA)	6mM	1.333	1.12	0.357	0.354	0.626	0.617
	8mM	1.587	1.503	0.389	0.374	0.709	0.676
	10mM	1.648	1.672	0.399	0.380	0.701	0.713
Salicylic acid (SA)	6mM	1.626	1.625	0.348	0.329	0.565	0.561
	8mM	1.458	1.406	0.354	0.378	0.610	0.675
	10mM	1.623	1.526	0.368	0.395	0.676	0.725
** Negative control		1.687	1.668	0.311	0.301	0.536	0.524
L.S.D. at5%:		0.128	0.113	0.034	0.031	0.0507	0.067

\* Positive control (untreated plants grown in infested soil), \*\*Negative control (untreated plants grown in un-infested soil)

**TABLE 9.** Effect of SA and NA on total phenols, total flavonoids, trigonelline and total carbohydrate of fenugreek plants grown in artificially infested/or un-infested soil by *S. rolfii* during 2018/2019 and 2019/2020 growing seasons

Treatments		Total phenols (g/100g)		Total flavonoids (g/100g)		Trigonelline (g/100g)		Total carbohydrates %	
		1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
		Plants grown in infested soil							
Nicotinic acid (NA)	6mM	1.34	1.50	0.72	0.77	0.411	0.389	35.73	36.16
	8mM	641.	1.663	0.79	0.82	0.264	0.257	39.14	37.79
	10mM	1.74	1.73	0.77	0.84	0.305	0.309	38.27	37.73
Salicylic acid (SA)	6mM	1.24	1.27	0.57	0.64	0.317	0.299	35.29	34.42
	8mM	1.77	1.63	0.65	0.67	0.246	0.255	37.56	1 36.4
	10mM	1.75	1.80	0.740	0.78	0.319	0.300	38.84	37.01
Topsin-M	2gm/L	1.55	1.50	0.54	0.67	0.308	0.302	38.36	37.91
*Positive control		1.54	1.49	1.50	0.42	0.245	0.241	33.03	33.10
Plants grown in un-infested soil									
Nicotinic acid (NA)	6mM	1.45	1.39	0.591	0.598	0.362	0.354	43.13	42.16
	8mM	1.53	1.47	0.672	0.679	0.435	0.409	45.64	44.27
	10mM	1.67	1.56	0.71	0.705	0.406	0.394	47.33	43.68
Salicylic acid (SA)	6mM	1.41	1.41	0.620	0.561	0.362	0.346	42.89	41.11
	8mM	1.46	1.53	0.705	0.648	0.398	0.363	43.71	42.83
	10mM	1.57	1.32	0.623	0.708	0.413	0.383	45.35	44.2°
** Negative control		1.49	1.33	0.466	0.531	0.339	0.325	40.28	40.35
L.S.D. at5%:		0.179	0.168	0.098	0.125	0.0202	0.023	2.19	1.296

\* Positive control (untreated plants grown in infested soil), \*\*Negative control (untreated plants grown in un-infested soil)

## Conclusion

From the results of the present study, we concluded that SA and NA at 10 mM promote plant growth characteristics and increase the marketable yield, also increase vegetative characteristics and active composition of fenugreek seed (total phenols, total flavonoids, and trigonelline), in most cases the differences between SA and NA were not significant. Furthermore, applying SA at 10mM was the superior treatment for controlling collar rot and root rot caused by *Sclerotium rolfsii* at all different growth stages of fenugreek plants. So, to obtain good results in fenugreek plants during the growing season, treating fenugreek plants with SA at a concentration 10mM as a potential inducer of systemic acquired resistance in fenugreek plants against *Sclerotium rolfsii* could be recommended.

*Conflict of Interest:* The authors declare that they have no conflicts of interest.

*Authors' contributions:* The corresponding author Hala F. Mohammed was involved in project conceptualization, execution, sample collection, analyzation, writing and editing of the manuscript. Doaa A. Imara was involved in execution, sample collection, and writing of the manuscript. All the authors read and approved the manuscript.

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### تقييم فاعلية حمض الساليسيليك وحمض النيكوتينيك في مكافحة عفن اليقافة وعفن الجذر المسبب له *Sclerotium rolfsii* وتحسين إنتاجية نبات الحلبة في مصر

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تعرضت الحلبة في السنوات الأخيرة للإصابة ببعض فطريات التربة مثل *Fusarium moniliforme* البادرات وعفن اليقافة وأعفان الجذور. وتسبب هذه الأمراض إلى تأخير النمو و السقوط لبعض النباتات المصابة. تم عزل خمس فطريات من أجزاء نباتات الحلبة المصابة طبيعياً. كان *Sclerotium rolfsii* أكثر الفطريات المعزولة تكراراً وأشد الفطريات قدرة مرضية. حيث أعطى *S. rolfsii* أعلى نسبة وشدة إصابة بسقوط البادرات وأعفان اليقافة والجذور من بين الفطريات المختبرة في إختبار العدوى الصناعية. أكدت النتائج أن حمض الساليسيليك وحمض النيكوتين بتركيز (10 ملي مول) يلية تركيز (8 ملي مول) أكثر فاعلية في استحثاث المقاومة ضد أمراض عفن اليقافة و الجذور، وقللت من شدة المرض وعدد النباتات الميتة، وانعكس ذلك على قياسات النمو وإنتاجية المحصول خلال موسمي النمو. كان لحمض الساليسيليك وحمض النيكوتين (10 ملي مول) تأثير معنوي في زيادة الصفات النباتية (الوزن الطازج والجاف للنبات (جم)) ومتوسط وزن القرون (جم) مقارنة بالنباتات الغير معاملة. علاوة على ذلك، أظهر التركيب الكيميائي للذور زيادة معنوية في محتوى الفينولات الكلية والفلافونويد والتريجونيلين والكاربوهيدرات. تشير النتائج أنه يمكن استخدام التطبيق الخارجى لحمض الساليسيليك بتركيز (10 ملي مول) بنجاح كمستحث للمقاومة المكتسبة وهو نهج واعد لمقاومة أمراض عفن اليقافة والجذور المتسبب عنه (*Sclerotium rolfsii*) وزيادة جودة وكمية محصول الحلبة؛ حيث يمكن استخدامها كاستراتيجية آمنة ضد هذه الأمراض.