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The physiological effectiveness of signal molecule salicylic acid and its nanoparticles on quality and quantity of *Pisum sativum* L. plants

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ABSTRACT

Nanotechnology is a novel technique used to enhance plant productivity and quality and has the capability to reduce the amount of using chemical substances to keep the safety of environment. Two field experiments were carried out in a private farm at Sharkia Governorate during the winter seasons of 2021/2022 and 2022/2023 to study effect of foliar spraying with salicylic acid (SA) at 100, 200 and 300 mgL^{-1} and its nanoform (SA NPs) at 25, 50 and 75 mgL^{-1} on both quality and economic characters of *Pisum sativum* L. plants. Results show that all applied treatments caused marked increases in vegetative growth parameters, photosynthetic pigments, chemical composition of leaf tissues, phenylalanine ammonia lyases (PAL) and tyrosine ammonia lyases (TAL), seed yield, yield components and chemical composition of the yielded seeds. It was noted the promoting effect of SA NPs was more pronounced than the effect of SA as bulk form. The most promised treatments were SA NPs at 50 mgL^{-1} followed by SA at 200 mgL^{-1}. Since, SA NPs at 50 mgL^{-1} significantly increased plant dry weight from 5.05 to 12.56 (2.49 times), total photosynthetic pigments from 1.230 to 2.377 (1.93 times), total carbohydrate of dry yielded seeds from 45.43 to 48.48 (1.06 times); starch content from 31.25 to 34.49 (1.10 times); total protein content from 23.01% to 25.43 (1.10 times), total phenolic content from 35.09 to 53.58 (1.52 times), flavonoid from 6.27 to 8.20 (1.30 times) and DPPH from 32.90 to 37.55 (1.14). Regarding the quantity of resultant yield, SA NPs at 50 mgL^{-1} significantly increased green yield/fed and weight of dry seed/fed by 3.87 times and 6.5 times respectively. This research clearly showed that SA NPs at low doses 50 mgL^{-1} has pronounced effect in increasing the quality and quantity of pea plant than the bulk form of salicylic acid at different concentration.

Keywords: *Pisum sativum,* nanotechnology, signal molecules, salicylic acid

1. Introduction

Phytohormones are signal molecules synthesized by plants at low concentrations (Jogawat *et al*. 2021) to regulate plant growth and development (Rhaman *et al.* 2020; Baltacıer *et al.* 2023). Salicylic acid (SA) is a phytohormone with a phenolic structure and having antioxidant potential (Sakhabutdinova *et al.* 2003). It is a potent endogenous signal molecule involved in intracellular communication pathways by regulating physiological and biochemical functions under normal or stressed conditions (Dawood and El-Awadi, 2020; Rhaman *et al*. 2020). Salicylic acid contributes in a number of processes, including promoting flowering, elevating CO_2 gas participation, regulating the movement of photo materials, gas exchange, boosting protein synthesis, and defense mechanisms to reduce ROS formation (Hasanuzzaman *et al.* 2015; Kedir, 2023). In addition, salicylic acid enhances plant development by promoting ion uptake and transport (Khan *et al.* 2015), improving photosynthetic capacity and stomatal conductance (Maqsood *et al.* 2023); regulating the respirational activities, (Kong *et al.* 2021), enhancing nitrogen metabolism, antioxidant defense system, and water use efficiency

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(Khan *et al*. 2015; Naz *et al.* 2021; Yang *et al.* 2023). It is evidenced that application of SA is demonstrated to behave as a cell protectant, and reducing the effects of oxidative stress through enhanced cell antioxidant activity (Naeem *et al.* 2020; Sharma *et al.* 2020; Maqsood *et al.* 2023) that is responsible for eliminating the active oxygen species (ROS) generated by the oxidative stress and minimizing the electrolyte leakage determined by loss of the integrity of the membrane (Faried *et al.* 2017; Pai and Sharma 2022), thus preserving cell turgor and enhanced shoot-root growth and biomass (Khattak *et al.,* 2021; Shemi *et al*. 2021). Moreover, salicylic acid enhanced accumulation of secondary metabolites, nitrogen-containing compounds, sulfur-containing compounds, and biosynthesis pathways of other plant growth regulators (Khan *et al.* 2015; Rasheed *et al.* 2020). It is worthy to mention that salicylic acid at 200 mg L^{-1}reduced the negative effects of salinity via improving the vegetative growth criteria, IAA, photosynthetic pigments, grain output and its attributes, leaf osmolytes, carbohydrate content, flavonoids, phenolic content, B-carotene and lycopene, of the developed wheat grains (Sadak and Dawood, 2022). Notably, the effect of SA on cellular and molecular metabolism differs depending on the level of SA, plant species and circumstances of the growth (Baltacıer *et al.* 2023).

Nanoparticles (NPs) are microscopic particles with at least one dimension between 1 and 100 nm size range. They differ from their bulk counterparts in terms of their physical, chemical, and electrical characteristics, which provide new research opportunities in many areas of science, including agriculture. In recent years, the use of NPs has increased due to its unlimited potential, eco-friendly, economical applications and beneficial effects in decreasing the negative impacts of chemicals used in agriculture (Bratovcic *et al.* 2021; Emmanuel *et al.* 2023). Nanoparticles can be used as a modern agricultural tool because of their ability to cross cellular barriers. Since NPs have unique optical features, highly soluble, size dependent qualities, high surface-to-volume ratio, and easily absorbed by plants (Souri *et al.* 2017). NPs differ from the bulk material in terms of their physicochemical and mechanical characteristics. It may be because of their tiny size, increased surface area to volume ratio, slow release when interact with other particles or living systems, quickly translocation rates, etc., (Karamian *et al*. 2020). The physiological and morphological impacts of NPs differ by plant species, as well as growth stage, dosage, application approach, and exposure duration (Rizwan *et al.* 2017). NPs are absorbed by plants in a number of ways, notably through their roots and leaves. According to the mass flow/pressure flow concept, NPs penetrate plant via the stomata and transport by the phloem via differences in pressure between the leaves and roots (Turgeon, 2010). The NPs are delivered by phloem tubes through apoplastic and symplastic pathways to the appropriate site or organs such as roots, shoots, and fruit (Usman *et al.* 2020). As NPs enter the plant cell, they alter many plant processes, including germination, macro and micronutrients, antioxidant activity, chloroplast number, chlorophyll content, and photosynthesis (Cinisli *et al.* 2019). It is worthy to mention that application of nanosalicylic acid lonely showed promise effect in inducing role of herbicide and reduced the weed propagation (Talaat *et al.* 2022), and when uploaded on chitosan, significantly improved yield of maize (Kumaraswamy *et al.* 2019), and improved the grape plant yield under salinity stress (Aazami *et a*l. 2023).

Pea (*Pisum sativum* L.) is the most significant legume crop in the world which has a wide range of uses for human food and animal feed. Pea is a member of the Fabaceae family, and considered as one of the most appreciated vegetable crops cultivated in Egypt for local market and exportation. Peas are a rich in nutrients, containing 15 to 35 % protein and high amounts of the essential amino acids *i.e.* tryptophan and lysine. In addition, pea also contains high content of carbohydrates (Elzebroek and Wind, 2008). Pea is a rich source of nutrients and secondary metabolites (antioxidants) that required for humans health including vitamins E and C, β-carotene, flavonoids, phenolic compound, minerals, and organic acids (Hounsome *et al.,* 2008). Inter-simple sequence repeats (ISSR) marker is more reliable than the RAPD marker because of its highly discriminating, fast, simple, low cost. So, it is regarded as an effective technique for determining genetic relationships (Osman and Ali, 2021).
This work aimed to compare the role of salicylic acid as signal molecule and its nano form as a novel technique to enhance productivity and quality of pea plant grown under Egyptian climatic changes.

2. Materials and Methods

2.1. Material

Salicylic acid was purchased from Sigma-Adrich dissolved in 10 ml absolute ethanol and shaking for an hour at (25°C) with an ultrasonic power and frequency of 50 kHz. Moreover, Salicylic acid nano-

synthesis and Transmission Electron Microscopy of salicylic acid nanoparticles (SA NPs) were done according to Tahsin *et al.* (2021).

2.2. Experimental design

Two field experiments were carried out in a private farm at Sharkia Governorate during the winter seasons of 2021/2022 and 2022/2023 to study the effectiveness of salicylic acid and its nano- form foliar spraying on both quality and economic characters in *Pisum sativum* plants. Seeds of *Pisum sativum cv.* Master B was obtained from Agriculture Research Center, Giza, Egypt.

The physical and chemical characteristics of studied soil (Table 1) were carried according to Cottenie *et al.* (1982). The recommended doses of chemical nutrients (P_2O_5, K_2O, Ca) were added once by broadcasting during soil preparation. Nitrogen fertilizer in ammonium nitrate form was added in two doses at the first irrigation (25 DAS) and 30 days later. All of specified cultural practices were carried out as recommended, including main field preparation, irrigation, fertilization, weeds control and plant protection as the recommendation of Ministry of Agriculture and Land Reclamation (MALR). The experimental unit consisted of ten rows, four meter in length, 50 cm in width and 20 cm apart between plants.

However, during the two experimental seasons, foliar application with three concentrations of salicylic acid (100, 200 and 300 mgL^{-1}) and nano-salicylic (25, 50 and 75 mgL^{-1}) were applied twice after 40 and 50 days after sowing. Moreover, the experiment treatments were arranged in a randomized complete block design (RCBD) with four replicates.

Table 1: Characteristics of the empirical soil in Sharkia governorate, Egypt (Collective data of two seasons).

Physical characteristics					
Soil Texture	**Clay**	**Silt**	**Sand**	**EC(ds .m^{-1})**	**pH**
Clay **(%)**	50.2	38.3	11.6	0.46	7.49
Chemical characteristics					
Cations (meq .1^{-1})			**Anions (meq .1^{-1})**		
Ca	**Mg**	**Na**	**SO4**	**CL**	**HCO_3**
2.19	0.68	1.15	3.36	1.18	1.40
Macronutrient			**Micronutrient (mg .kg^{-1})**		
N (meq .1^{-1})	**P (ppm)**	**K (ppm)**	**Zn**	**Fe**	**Mn**
47.2	26.5	386	1.16	5.9	0.30

2.3. Collected data

After 60 days of sowing during vegetative growth parameters, plant samples were collected for determination of morphological characteristics (plant height (cm), number of leaves and branches/plant, fresh and dry weight of plant (g)). Dry weight was measured after drying plant sample in oven for 48h at 50^{o}C. Fresh leaves were collected for determination photosynthetic pigments, phenylalanine ammonia lyases (PAL) and tyrosine ammonia lyases (TAL). While dry leaves were used to determine, total carbohydrate, total soluble sugar, free amino acids, proline content, and protein content.

At harvest time, ten plants from each treatment were chosen randomly to measure the number of pods/plant, number of seeds/pod, average pod weight, weight of both green and dry yield/fed. (kg). The yielded dry seeds were used to determine total carbohydrate, total soluble sugar, starch, protein content, phenolic content, flavonoid, and DPPH.

2.4. Biochemical analyses

Photosynthetic pigments were determined according to Li and Chen (2015). Total carbohydrates content was determined according to Albalasmeh (2013). Total soluble sugars (TSS) were determined by the method of Mecozzi (2005). Starch content was determined according to Chow and Landhausser (2004). Polysaccharides were calculated by the difference between total carbohydrate content and total soluble sugars. Free amino acid was determined by Tamayo and Pedrol (2001). Proline content was determined by Kalsoom *et al.* (2016). Total protein was determined according to Pedrol and Tamayo (2001). PAL activity in the partially purified enzyme extracts was assayed by an adaptation of the

method reported by McCallum and Walker (1990). TAL activity of enzyme extracts was measured using a spectrophotometric assay of Montero *et al. (*1998). Total phenolic content was determined by Gonza'lez *et al.* (2003). Flavonoid content was determined by Chang *et al.* (2002). The free radical scavenging activity by 2, 2,- diphenyl-2-picryl-hydrazyl (DPPH) method was determined according to Gyamfi *et al.* (1999).

2.5. Extraction of DNA and PCR procedures

Soft, fresh and young leaves of *Pisum sativum* plants under study were used to isolate and extract the genetic material (DNA) using CTAB according to Khaled and Esh (2008) modified method.
A total of 10 ISSR primers were tried, but 5 ISSR primers with positive results Table (2) were used in this study. However, the primers were chosen upon a previously used with different plant groups (fenugreek, bean, lupine, checkpea, surghum and soybean) and the primers with high polymorphism rate were selected.

In order to perform PCR based analysis, it was carried out within 15 µl reaction volumes containing 1 µl DNA of plant, 7.5 µL Master Mix, 1 µL template DNA and 1 µL primer.
The program of PCR was 94°C for 5 min forinitial denaturation, 94°C for 1 min (40 cycles), 37ºC for 1 min, 72ºC for 2 min and final extension at 72ºC for 10 min. As soon as, the amplified products were electrophoresed on 1.5 % agarose gel in 1×TAE buffer. Then ethidium bromide was used to stain the gel that photographed using gel documentation system.

Table 2: The positive ISSR primers with their sequences that used in this study.

	Primer	Primer Sequence (5\ ⟶ 3\)
1	**$(AG)_8$ CG**	**AGAGAGAGAGAGAGAGCG**
2	**$(AG)_8$ TG**	**AGAGAGAGAGAGAGAGTG**
3	**$(CA)_8$ GC**	**CACACACACACACACAGC**
4	**$(AGC)_6$ G**	**AGCAGCAGCAGCAGCAGCG**
5	**$(AG)_9$ G**	**AGAGAGAGAGAGAGAGAGC**

2.6. Data analysis

Amplified DNA amplicons using ISSR marker were classified as absent (0) or present (1) and total number of bands (TB), polymorphic bands (PB), monomorphic bands (MB), unique bands (UB) and percentage polymorphism (PB%) were calculated.

2.7. Statistical analysis

Analysis of variance was used to statistically examine the average data average of two seasons. The differences between means were calculated using (LSD) at 5% according to Silva and Azevedo (2016).

3. Results

Table (3) shows that all applied treatment significantly increased the vegetative growth parameters under investigation except SA at 100 mgL^{-1} that showed non significant increases in plant height, number of leaves and branches/plant relative to control. It was noted the effect of SA NPs was more pronounced than the effect of SA. Since, the highest significant increases in growth parameters was due to SA NPs (50 mgL^{-1})> SA NPs (75 mgL^{-1}) > SA (200 mgL^{-1}) > SA (300 mgL^{-1}). Notably, SA NPs at 50 mgL^{-1} significantly increased plant fresh weight from 30.78 g to 76.62 g (2.49 times), and plant dry weight from 5.05 to 12.56g (2.49 times).

Figure (1) shows that all applied treatments caused marked increases in all components of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoid) relative to control. It was noted the effect of SA NPs was more pronounced than the effect of SA. Since, the highest significant increases in total photosynthetic pigments was recorded due to SA NPs at 50 mgL^{-1} followed by SA at 200 mgL^{-1}. Notably, SA NPs at 50 mgL^{-1} significantly increased total photosynthetic pigments from1.230 mg/g to 2.377 mg/g (1.93 times), and SA at 200 mgL^{-1} significantly increased total photosynthetic pigments from 1.230 mg/g to 2.331 mg/g (1.89 times).

Table 3: Effect of salicylic acid (SA) and its nanoform (SA NPs) on vegetative growth parameters of pea.

Treatments	Plant height (cm)	Number of branches/plant	Number of leaves/plant	Plant fresh weight (g)	Plant dry weight (g)
Control	41.00	1.67	12.67	30.78	5.05
SA (100 mgL^{-1})	45.33	1.67	18.67	47.33	7.76
SA (200 mgL^{-1})	47.67	3.33	28.00	69.60	11.41
SA (300 mgL^{-1})	47.67	2.83	26.00	60.36	9.89
SA NPs (25 mgL^{-1})	46.33	2.67	21.00	55.82	9.15
SA NPs (50 mgL^{-1})	52.33	4.00	33.33	76.62	12.56
SA NPs (75 mgL^{-1})	50.33	3.33	28.33	70.52	11.56
LSD at 5%	**5.11**	**1.14**	**6.44**	**3.98**	**0.65**

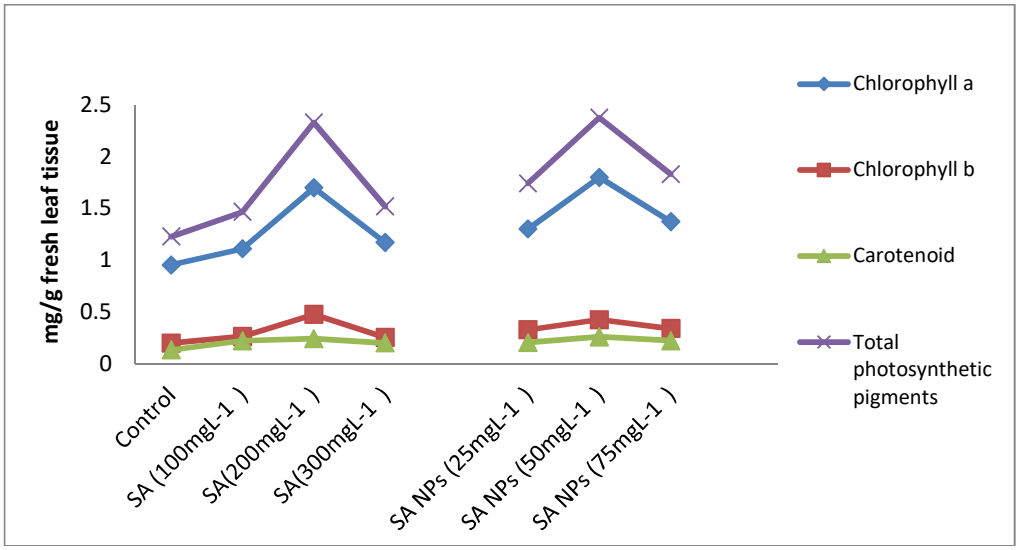

Fig. 1: Effect of salicylic acid (SA) and its nanoform (SA NPs) on photosynthetic pigments of fresh leaf tissues of pea
(LSD for chlorophyll a is 0.57; chlorophyll b is 0.19; carotenoid is 0.10; photosynthetic pigments is 0.81)

Table (4) shows that all applied treatments significantly increased total carbohydrate content, total soluble sugar and polysaccharides of dry leaf tissues of pea relative to control. It is obvious that SA at 200 mgL^{-1} showed the highest significant increase of total carbohydrate content and polysaccharides followed by SA NPs at 50 mgL^{-1} accompanied by the lowest significant increase in total soluble carbohydrate relative to control. SA at 200 mgL^{-1} significantly increased total carbohydrate content from 24.10 % to 31.84% (1.32 times).

Table (4) shows that all applied treatments significantly increased total free amino acids, proline and protein content of dry leaf tissues of pea relative to control. It was noted that SA NPs at 50 mgL^{-1} showed the highest significant increase of total free amino acids followed by SA at 200 mgL^{-1} and SA at 300 mgL^{-1}. On the other hand, the highest significant increases in proline and protein content were recorded due application of SA at 200 mgL^{-1} > SA at 300 mgL^{-1} > SA NPs at 50 mgL^{-1}. SA at 200 mgL^{-1} significantly increased total protein content 18.55% from % 24.93 to % (1.34 times).

Regarding PAL and TAL enzymes, all applied treatments significantly increased both enzymes relative to control (Figure 2). The highest significant increase in PAL enzyme was recorded due to SA at 200 mgL^{-1} > SA NPs at 50 mgL^{-1} > SA NPs at 75 mgL^{-1}. Whereas, SA NPs at 75 mgL^{-1} showed the highest significant increases of TAL enzyme followed by SA at 200 mgL^{-1} relative to control.

Table 4: Effect of salicylic acid (SA) and its nanoform (SA NPs) on some biochemical composition of leaf tissues of pea

Treatments	Total carbohydrate %	Total soluble sugars %	Polysaccharides %	Free amino acids	Proline	Total protein %
				mg/100 g dry tissue		
Control	24.10	1.49	22.24	231.50	35.09	18.55
SA (100 mgL^{-1})	27.76	2.18	25.58	241.02	46.61	21.77
SA (200 mgL^{-1})	31.84	1.85	30.35	250.53	53.88	24.93
SA (300 mgL^{-1})	30.84	2.43	28.41	250.52	50.67	24.36
SA NPs (25 mgL^{-1})	28.81	2.27	26.54	247.18	42.30	21.26
SA NPs (50 mgL^{-1})	31.05	2.12	28.93	259.02	50.58	23.90
SA NPs (75 mgL^{-1})	28.87	2.39	26.48	249.19	43.76	22.71
LSD at 5%	**0.89**	**0.05**	**0.09**	**3.19**	**0.66**	**0.51**

Table (4) shows that all applied treatments significantly increased total free amino acids, proline and protein content of dry leaf tissues of pea relative to control. It was noted that SA NPs at 50 mgL^{-1} showed the highest significant of total free amino acids followed by SA at 200 mgL^{-1} and SA at 300 mgL^{-1} . On the other hand, the highest significant increases in proline and protein content were recorded due application of SA at 200 mgL^{-1} >SA at 300 mgL^{-1} > SA NPs at 50 mgL^{-1}. SA at 200 mgL^{-1} significantly increased total protein content 18.55% from % 24.93 to % (1.34 times).

Regarding PAL and TAL enzymes, all applied treatments significantly increased both enzymes relative to control (Figure 2). The highest significant increase in PAL enzyme was recorded due to SA at 200 mgL^{-1} >SA NPs at 50 mgL^{-1} >SA NPs at 75 mgL^{-1}. Whereas, SA NPs at 75 mgL^{-1} showed the highest significant increases of TAL enzyme followed by SA at 200 mgL^{-1} relative to control.

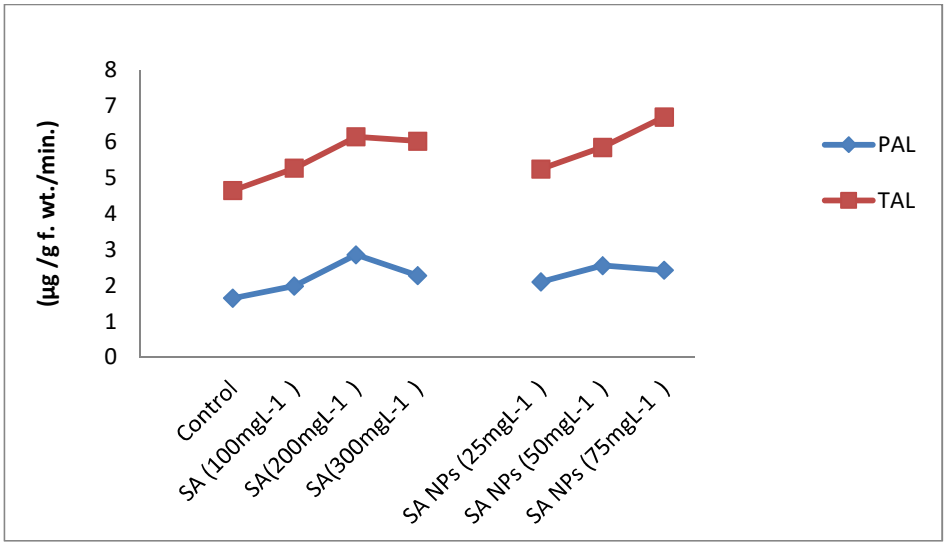

Fig. 2: Effect of salicylic acid (SA) and its nanoform (SA NPs) on PAL (µg cinnamic acid/f fresh wt/min) and TAL ((µg p-coumaric acid/f fresh wt/min) of leaf tissues of pea. (LSD for PAL is 0.07; for TAL is 0.24**)**

Table (5) shows that all applied treatments caused significant increases in number of pods/plant except SA at 100 mgL^{-1} and SA NPs at 25 mgL^{-1}. In addition, all applied treatments caused significant increases in number of seeds/pod except SA at 100 mgL^{-1}, SA at 300 mgL^{-1} and SA NPs at 75 mgL^{-1}. On the other hand, average pod weight, weight of green yield/fed. and weight of dry seeds/fed. (kg) were significantly increased by all applied treatments. It was noted the effect of SA NPs was more pronounced than the effect of SA. The enhancement effect of SA NPs at 50 mgL^{-1}> SA NPs at 75 mgL^{-1}> SA at 200 mgL^{-1}. Since, SA NPs at 50 mgL^{-1} significantly increased green yield/fed and weight of dry seed/fed by 3.87 times and 6.5 times respectively.

Table 5: Effect of salicylic acid (SA) and its nano form (SA NPs) on seed yield and yield components of pea

Treatments	Number of pods/plant	Number of seeds /pod	Average pod weight (g)	Green yield/fed. (kg)	Weight of dry seeds/fed. (kg)
Control	12.00	7.33	2.75	2640.00	403.20
SA (100mgL^{-1})	13.30	7.67	3.68	3915.52	957.60
SA (200mgL^{-1})	18.33	10.67	4.02	5894.93	1539.72
SA (300mgL^{-1})	16.35	8.00	3.70	4839.60	1281.84
SA NPs (25mgL^{-1})	15.33	9.00	3.69	4525.416	1312.25
SA NPs (50mgL^{-1})	26.67	9.76	4.79	10219.94	2624.33
SA NPs (75mgL^{-1})	20.00	8.02	4.52	7232.00	1696.00
LSD at 5%	**4.28**	**0.76**	**0.32**	**1739.60**	**339.57**

Table (6) shows that all applied treatments significantly increased total carbohydrate content, total soluble sugars, starch content, total protein content, total phenolic compound, flavonoid and DPPH %. It was noted the effect of SA NPs was more pronounced than the effect of SA. The most promised treatments were SA NPs at 50 mgL^{-1} followed by SA at 200 mgL^{-1}. It was noted that SA NPs at 50 mgL^{-1} significantly increased total carbohydrate from 45.43% to 48.48% (1.06 times); starch content from 31.25% to 34.49% (1.10 times); total protein content from 23.01% to 25.43% (1.10 times), total phenolic content from 35.09 to 53.58 (1.52 times), flavonoid from 6.27 to 8.20 (1.30 times) and DPPH from 32.90% to 37.55% (1.14).

Table 6: Effect of salicylic acid (SA) and its nanoform (SA NPs) on some chemical composition of the dry yielded seeds of pea

Treatments	Total carbohydrate %	Total soluble sugars %	Polysaccharides %	Starch content %	Total protein %	Total phenolic content (mg/100 g dry wt)	Flavonoid (mg/100 g dry wt)	DPPH%
Control	45.43	12.90	32.53	31.25	23.01	35.09	6.27	32.90
SA (100mgL^{-1})	46.52	13.94	32.58	32.05	23.87	46.61	7.30	35.01
SA (200mgL^{-1})	47. 43	14.47	32.96	34.12	25.40	50.88	8.12	37.51
SA (300mgL^{-1})	47.02	14.19	32.83	33.43	24.06	50.67	8.08	36.72
SA NPs (25mgL^{-1})	47.07	13.55	33.52	33.05	24.57	42.30	7.43	35.89
SA NPs (50mgL^{-1})	48.48	14.09	34.39	34.49	25.43	53.58	8.20	37.55
SA NPs (75mgL^{-1})	47. 37	14.02	33.35	33.88	24.78	43.76	8.20	36.71
LSD at 5%	**0.45**	**0.64**	**0.50**	**0.46**	**0.38**	**0.65**	**0.21**	**0.48**

ISSR- Molecular Markers

Recorded data in Tables (2 and 7) showed the summary effects of both salicylic acid (SA) and its nano-particles (SA NPs) on reproducible DNA fragments of Pea plants. In this study 10 ISSR primers (Table 1) were used for polymorphism screening, out of which only 5 primers were found polymorphic. However, multiple fragments with different molecular weights were detected using these primers, and the reproducible fragments distributed between polymorphic, monomorphic and unique bands.

Table 7: Effect of salicylic acid (SA) and its nanoform (SA NPs) on ISSR- markers of pea plants

	Primer	Marker size (bp)	Amplified bands TB	PB	MB	UB	PB %
IS -01	**$(AG)_8$ CG**	**1213.66 – 226.86**	16	7	3	6	81.25
IS -02	**$(AG)_8$ TG**	**1202.16 – 191.17**	16	5	4	7	75.00
IS -03	**$(CA)_8$ GC**	**846.73 – 218.72**	15	5	4	6	73.33
IS -04	**$(AGC)_6$ G**	**931.69 – 225.67**	17	5	3	9	82.35
IS -05	**$(AG)_9$ G**	**1268.24 – 248.04**	11	4	2	5	81.82
	Total		**75**	**26**	**16**	**33**	**-**
	Average		**15.00**	**5.2**	**3.2**	**6.6**	**78.75**

It was noticed that the total detected bands (TB) were 75 bands that distributed as 26 were polymorphic (PB). The highest level of polymorphism could be observed with primers IS-04 primer

that showed (82.35%) polymorphism, while the lowest polymorphism was 73.33% with primer IS-03 (Table 8).

Moreover, the detected bands were varied in number, polymorphism and range of its molecular weights between used ISSR primers. With regard to primer IS-04, 17 bands were detected with this primer and molecular weights of these bands ranged between (931.69 – 225.67bp), moreover, it distributed as 3 monomorphic bands (MB), 9 unique bands (UB) and 5 polymorphic bands (PB) with 82.35% polymorphism. Meanwhile, there were 16 bands with molecular weights (1213.66 – 226.86bp) and 81.25% polymorphism were detected usingIS-01 primer, and distributed as 3 (MB), 6 (UB) and 7 (PB). Moreover, there were 16 bands with molecular weights (1202.16 – 191.17bp) and 75.00% polymorphism were detected usingIS-02 primer, and distributed as 4 (MB), 7 (UB) and 5 (PB). Table (8)

On the other hand, the lowest total amplified bands, polymorphic bands and unique and (11 bands, 4 bands, 2 bands with polymorphism 81.82%), respectively, were scored with IS-05 primer with molecular weights ranged between (1268.24 – 248.04). Table (7) and Fig. (3).

With regard to Table (7) that present a general idea about the reproducible bands detected using previous five ISSR primers. However, next table (Table 8) draws the attention to number, size, type and conjugative reproducible bands that were detected by each primer separately. Moreover, there were some bands which have the same molecular weight and these called polymorphic bands and this conjunction due to the effect of the treatments (Table 8).

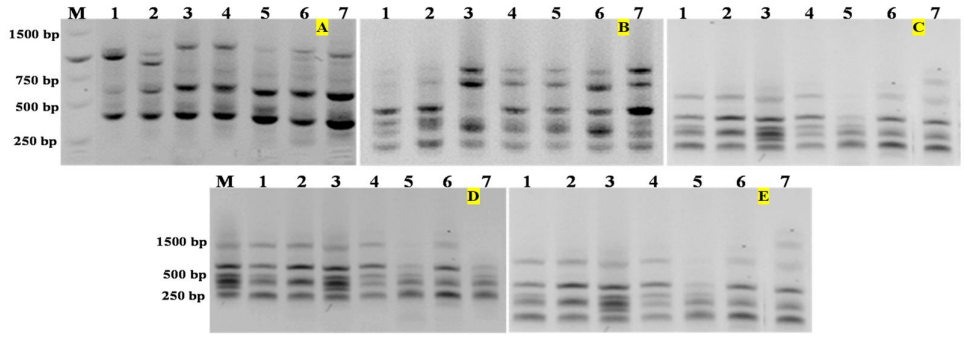

Fig. 3: Effect of salicylic acid (SA) and its nanoform (SA NPs) on ISSR- markers of pea plants.
A=IS-1, B=IS-2, C=IS-3, D=IS-4, E=IS-5 M=DNA Marker;
1=control; 2=SA (100mgL^{-1})**; 3= SA** (200mgL^{-1})**; 4= SA**(300mgL^{-1})**; 5= SA NPs** (25mgL^{-1})**;**
6= SA NPs (50mgL^{-1})**; 7**= **SA NPs** (75mgL^{-1})**.**

Table 8: Effect of foliar spraying with salicylic acid (SA) and its nanoform (SA NPs) on ISSR- markers of pea plants.

MW	Cont.	SA 100	SA 200	SA 300	SA NPs 25	SA NPs 50	SA NPs 75	Polymorphism
				IS-01				
1213.66	0	0	1	1	0	0	0	BP
1145.28	0	1	0	0	0	0	0	UB
1056.93	0	0	0	0	0	1	1	BP
1019.88	1	0	0	0	0	0	0	UB
975.39	0	0	1	0	0	0	0	UB
920.44	0	0	0	1	0	0	0	UB
906.18	0	1	0	0	0	0	0	UB
855.13	0	0	1	0	1	0	0	BP
794.46	1	0	0	0	0	0	0	UB
753.06	0	0	0	1	1	1	1	BP
620.25	1	1	1	1	1	1	1	MB
487.49	1	1	1	1	1	1	1	MB
378.05	1	1	1	1	1	1	1	MB
303.83	0	1	1	0	0	0	0	BP
278.52	1	1	0	1	1	1	1	BP
226.86	0	0	0	1	1	0	1	BP

IS-02								
1202.16	0	1	0	0	0	0	0	UB
1051.95	0	1	1	0	0	0	1	BP
918.39	1	0	0	0	0	0	0	UB
879.10	0	1	0	0	0	0	0	UB
798.11	0	0	1	1	1	1	1	BP
631.13	1	1	1	1	1	1	1	MB
595.85	1	1	0	0	0	0	0	BP
537.23	0	0	0	1	1	0	0	BP
497.94	0	0	1	0	0	0	0	UB
483.26	0	0	1	0	0	1	1	BP
430.74	1	1	1	1	1	1	1	MB
386.58	0	0	1	0	0	0	0	UB
317.90	1	1	1	1	1	1	1	MB
235.70	1	1	1	1	1	1	1	MB
212.51	0	0	0	0	1	0	0	UB
191.17	0	0	0	0	0	0	1	UB
IS-03								
846.73	1	1	0	0	0	0	0	BP
832.88	0	0	0	0	1	1	1	BP
696.21	0	0	0	0	1	0	0	UB
680.61	0	0	0	0	0	1	1	BP
629.36	0	1	1	1	0	0	0	BP
607.69	0	0	0	0	0	0	1	UB
580.76	0	0	0	0	1	0	0	UB
564.25	0	0	0	0	0	1	0	UB
499.67	1	1	1	1	0	1	1	BP
381.48	1	1	1	1	1	1	1	MB
323.51	0	0	0	0	1	0	0	UB
254.21	1	1	1	1	1	1	1	MB
286.48	1	1	1	1	1	1	1	MB
309.17	0	0	0	1	0	0	0	UB
218.72	1	1	1	1	1	1	1	MB
IS-04								
931.69	0	0	0	0	0	0	1	UB
876.36	0	0	0	0	0	1	0	UB
720.73	0	0	1	0	0	0	0	UB
698.31	0	1	0	0	0	0	0	UB
677.93	1	0	0	0	0	0	0	UB
610.56	0	0	0	1	0	0	0	UB
538.07	1	1	1	1	0	1	1	BP
479.84	0	0	0	0	1	0	0	UB
464.91	1	0	0	0	0	1	0	BP
458.53	0	1	0	0	0	0	0	UB
431.30	0	0	1	1	1	0	0	BP
379.34	1	1	1	1	1	1	1	MB
314.45	1	0	1	1	0	0	0	BP
300.49	0	0	0	0	1	1	0	BP
291.72	0	1	0	0	0	0	0	UB
274.39	1	1	1	1	1	1	1	MB
225.67	1	1	1	1	1	1	1	MB
IS-05								
1268.24	0	0	0	1	0	0	0	UB
1043.58	0	0	0	1	0	0	0	UB
973.09	0	0	1	0	0	0	0	UB
960.80	0	0	0	0	0	1	0	UB
812.69	0	0	0	0	0	0	1	UB
793.96	1	1	0	1	0	0	0	BP
757.79	0	0	1	0	0	1	0	BP
597.68	1	1	1	1	0	1	0	BP
458.59	1	1	1	1	1	1	1	MB
377.36	1	1	1	1	1	1	1	MB
248.04	1	1	1	1	0	1	0	BP

4. Discussion

Salicylic acid under different conditions regulated different physiological and biochemical processes, like flower induction, stomatal conductance, and transpiration (Rivas-San Vicente and Plasencia, 2011) photosynthesis and protein synthesis (Hayat *et al.* 2010), modifications to the hormonal status (Abreu and Munne´-Bosch, 2009) and lessens plant oxidative damage, which promotes growth (Farhadiand Ghassemi-golezani, 2020; Silva *et al.* 2023). In addition, SA plays crucial roles in improving plant growth through promoting cell division and cell expansion and enhancing nutrients uptake by the plants (Ahmad *et al.* 2023). Since, the accumulation of biomass noted in plants subjected to SA concentration at 1.6mM, even when exposed to the salinity stress, is probably attributed to the role of salicylic acid in boosting the production of proteins, lignin, and carbohydrates (Shao *et al.* 2018). Moreover, Badr and Fayed (2020) studied the effect of foliar application with SA and humic acid on pea and stated that foliar applications with (humic or salicylic) acids caused a significant increment in growth, yield and quality of peas.

Also, exogenous application of SA decreased the deleterious effects of salinity stress on mung bean through the improvement of plant growth, photosynthesis, and antioxidant system (Morsi *et al.* 2018). Recent evidence suggests that application of SA enhanced concentration of photosynthetic pigments (Rasheed *et al*. 2021; Silva *et al.* 2021), positively affected on chloroplast structure (Uzunova and Popova, 2000), regulated stomatal closure (Melotto *et al.* 2006), stimulated activity of enzymes such as RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) and carbonic anhydrase (Slaymaker *et al.* 2002) and enhanced the values of net CO_2 assimilation rate (Sumaira *et al.* 2014). Recently, Alam *et al.* (2023) stated that application of SA on wheat plant at lower concentration enhanced pigmentation and a reduced transpiration rate, thus evoking stomatal closure. Uzunovaand Popova (2000) stated that lower concentration of SA (10 lM) improves the photosynthetic net CO_2 assimilation in mustard seedlings. On the other hand, treatment with SA (systemic acquired resistance) caused accumulation of osmolytes such as proline, soluble proteins, glycine-betaine, and soluble sugars, etc. (Misra and Misra, 2012) to maintain osmotic homeostasis (Khan *et al*. 2015; Abdi *et al.* 2022). Since, the stimulation effect of SA on the production of soluble sugars and proteins was related to an increase in the photosynthetic pigments which in turn increased the carbohydrate content (El-Shraiy and Hegazi, 2009). The enhancement of proteins content is possibly due to the role of SA in inducing the nitrate reductase activity and protein kinase synthesis. This is linked with improved management of a number of metabolic processes, such as cell division and differentiation, as well as increased antioxidant activity (Fahad and Bano, 2012). SA maintains the metabolic pathways and transduction signaling that regulates proline biosynthesis, in order to preserve ionic homeostasis and low cytosolic potential (Faried *et al.* 2017). Therefore, the increase in proline content by SA administration boosts the osmotic management, and defenses of plants by stimulation of the integrity of the membrane, ROS scavenging, protein and enzymatic activities (Fahad and Bano, 2012; Khaleghi *et al.* 2019). It was noted by La *et al*. (2019) that SA application *on Brassica napus* plant grown under water deficit conditions showed 1.4 times elevation in the expression of proline biosynthetic genes, as1-pyrroline-5-carboxylate synthetase (P5CS1 and P5CS2) and pyrroline-5-carboxylate reductase (P5CR). It was confirmed that salicylic acid participates in the modulating several kinds of biochemical processes in plants and boosted the growth and crucial quality traits as total sugar (Farahat *et al.* 2007), protein content (Rahman *et al.* 2008) and total free proline (El-Khallal *et al.* 2009).

Seed-priming with salicylic acid increased overall plant performance of pea (*Pisum sativum*), stimulated photosynthesis-related attributes, activated antioxidant defense system and accumulation of osmolytesas proline and soluble sugars (Ahmad *et al*. 2020).

It is worthy to mention that SA is synthesized from the chorismate through two independent pathways, isochorismate synthase- and phenylalanineammonia-lyase–dependent pathways (Dempsey and Klessig, 2017). Since, salicylic acid activated several enzymes as nitrite reductase, phenylalanine ammonia-lyase, glucanase, etc that increased plant development (Ahmad *et al.* 2023). Previous research has shown that the SA treatment increased the activity of the PAL enzyme (Danaee *et al.* 2013).

It is worthy to mention that SA plays a vital role in biochemical and physiological processes along the entire life span of the plant (Rivas-San Vicente and Plasencia, 2011), such as increase photosynthetic processes (Fariduddin *et al.* 2003), absorption of ions (Simaei *et al.* 2012), production of soluble sugar and soluble protein (Chandra *et al*. 2007), and flowering (Wada and Takeno, 2010) all of which can directly or indirectly influence the crop yield.

SA application exerted beneficial role on flowering (Rivas-SanVicente and Plasencia, 2011), that eventually increased the total number of fruits (Ondrašek *et al.* 2007) and enhance the fruit production.

The increases in crop yield due to SA treatment has been related to promote cell division and cell enlargement (Hayat *et al*. 2010), regulate plant hormones such as auxin, cytokinin and ABA (Orabi *et al.* 2015) and enhance net photosynthetic rate, internal CO_2 concentration and water use efficiency (Fariduddin *et al.* 2003). Furthermore, the positive impact of SA on seed yield may be attributed to translocation of even more photo-assimilates to seeds during seed filling, hence boosting seed weight (Dawood *et al.* 2012; Sadak *et al*. 2013). Recently, application of SA increased wheat yield and its attributes as well as protein and carbohydrate content of grains as mentioned by Fayez *et al*. (2023).

Besides, Orabi *et al.* (2015); Rasheed *et al.* (2020) concluded that application of SA increased secondary metabolites with antioxidant activity as total phenolics and other. Previously, SA application on sweet cherry at three fruit developmental stages-boosted the fruit weight and increased the content of total phenolics compound, total anthocyanins, and antioxidant activity (Gimenez *et al.* 2014). Frequently, it has been suggested that SA directly affects on the activity of antioxidant enzymes that encourage the synthesis of metabolites and present in fruits and vegetables, particularly those with nutritional value in the final product (Huang *et al.* 2008).

Likewise, application of SA increased flavonoid accumulation in a number of plant species (Tounekti *et al.* 2013). Ilahy *et al.* (2011) observed a good significant relationship between antioxidant activity and main antioxidants (flavonoids total phenolics and vitamin C). Application of SA on tomato plants could be a viable way to increase tomato productivity, fruit quality characteristics, and health-beneficial substances (such as vitamin C, phenolic compounds, and flavonoids with antioxidant activity), because SA plays a variety of regulatory roles in plant metabolism (Pai and Sharma, 2022).
Regarding SA NPs, it was noted that NPs have a high surface/volume ratio, and high surface energy that improved their biochemical activity (Juárez-Maldonado *et al.* 2019). NPs can interact with plants and activate molecular pathways very quickly (Ahmad *et al.* 2020) and produce more antioxidant molecules (Costa and Sharma, 2016). It has been shown that NPs currently display a moderately broad spectrum of actions i.e. increasing water uptake in seeds, stimulation of photosynthesis, metabolism of starch reserves, modification of phytohormone levels, modulation of oxidative stress or increasing nutrient uptake (Khalid *et al.* 2022). It is worthy to mention that application of NPs have demonstrated great potential for usage as inducers of phytohormone biosynthesis, (Tripathi *et al.* 2022; García-Ovando *et al.* 2022) and can increase growth, biomass output, and yield of several agricultural crops (Khan and Upadhyaya, 2019).

It has been reported that NPs may regulate the responses of plants to stress, by influencing the levels of hormones, homeostasis of ion, activity antioxidant enzyme, expression of gene, and defense system functions (Zulfiqar and Ashraf, 2021; Khalid *et al.* 2022). A few studies have shown that NPs can penetrate the seed coat and improve the ability of the seed to absorb and use water. This in turn stimulates the enzymatic system, which improves germination and seedling growth (Banerjee and Kole, 2016). Nanotechnology has the ability to increase efficiency of plant photosynthesis via altering activity the enzymatic involved in the C3 cycle, and regulating the content of photosynthetic pigments responsible for plant growth (Lowry *et al*. 2019). Exogenous application of NPs on cucumber plants - during the growth phase-promoted rubisco activase activity, photosynthetic rate and, chlorophyll content, which led to an increase in plant dry biomass (Ghani *et al.* 2022). Application of NPs at high concentrations exerts a negative effect on photosynthesis, resulting to retardation of plant growth or death (Tripathi *et al.* 2017).

It has been shown that NPs can enhance the production of photosynthetic pigment, regulate the redox status, promote glucose metabolism, increase antioxidant defense activities, and modulate plant hormone signaling to increase defenses of plants against abiotic stress (Liu *et al.* 2023). It was further noted that application of NPs on soybean as foliar spraying increased the seed yield due to enhanced photosynthesis (Linh *et al*. 2020). It was noted that carbon nanotubes (CNTs) treatment increased seed germination and antioxidant content of maize (Liu *et al.* 2016). SA nanosphere increased the activities of peroxidase, chitinase, phenylalanine ammonia-lyase, polyphenol oxidase, and enhance immunity of plant to inhibit Phytophthora *nicotianae* (Feng *et al.,* 2020). The chitosan salicylic acid NPs act as a bio stimulant that can be effectively used to increase the yield of grape under salt stress (Aazami *et al.* 2023). Salicylic acid chitosan NPs significantly boosted maize yield by improving grain yield/plant (Kumaraswamy *et al.* 2019). Talaat *et al.* (2022) proposed the possibility of using the antioxidant nano-

salicylic acid with the herbicide bentazon to lessen the herbicide's negative effects on the growth and yield of *Pisum sativum* plants while maintaining the herbicide's selectivity and efficacy against weeds, and to significantly reduce weed propagation. Application of zinc-chitosan-salicylic acid nanoparticles at 100 mg L^{-1} has the capacity to modulate plant osmotic status, boost synthesis of osmoprotectants, stimulate ROS scavenging enzymes for preserving membrane integrity and cellular protection, and increase yield increment during water deficit (Das *et al.* 2023).

4.1. ISSR- Molecular Markers

Regarding changes in the reproducible ISSR-DNA fragments that detected as a result to the effect of foliar spraying with salicylic acid (SA) and its nano-particles (SA NPs) on pea plants were shown in Tables (8 and 9) and illustrated in Figure (3). Several studies showed that there are number of reports on characterization and determination of genetic diversity in pea. Shebl *et al.* (2018), used ISSR markers with four (*Vicia faba* L.) genotypes (Giza 716, Sakha 1, Sakha 3, and Sakha 4) to determine which contains high level of phenylalanine, moreover, they found that Sakha 3 genotype recorded the highest number of ISSR marker bands which could be correlated to the high accumulation of phenylalanine.

Al-Musawi *et al.* (2020) studied the relationship between five different genotypes of pea that cultivated in Iraqi. They used the ISSR markers to detect the genetic polymorphism among these genotypes. Osman and Ali (2021) studied the genetic relationship of some *Pisum sativum* subspecies using different molecular markers such as ISSR. Mohamed *et al.* (2023) evaluated the genetic relationships of some endangered Tunisian peas that adapted to arid regions by ISSR Markers and they recorded that the information collected from this work can help pea breeders to implement a selection program that improves the distribution of this crop in Turkey and in the arid regions of Southern Tunisia.

5. Conclusion

Nanotechnology is a novel technique to enhance plant productivity and quality and has the capability to reduce the amount of using chemical fertilizer to keep the safety of environment. This research clearly showed that SA NPs at low doses 50 mgL^{-1}has pronounced effect in increasing the quality and quantity of pea plant than the bulk form of salicylic acid at different concentration.

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