



Response of Pea Plants (*Pisum sativum*) to Nano-Salicylic Acid and Herbicides

I. M. Talaat, I. M. El-Metwaly, M. G. Dawood, M. M. El-Awadi*, K. G. El-Rokiek

Botany Department, Agriculture and Biological Institute, National Research Centre, 33 El Bohouth st P.O. 12622, Dokki, Giza, Egypt

*Corresponding author, Email address: el_awadi@yahoo.com

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*Corresponding author
el_awadi@yahoo.com

Abstract

Two field experiments were conducted in the green house of the National Research Centre, Dokki, Cairo, Egypt during two winter seasons (2019/2020 - 2020/2021) to study the effect of nano-salicylic acid and the herbicide bentazon and the interaction between them on the growth and yield of pea plants. The herbicide was applied at the recommended dose and at half of the recommended dose (1 L/fed. and ½ L/fed.). Nano-salicylic acid treatments were (0, 50 and 100 ppm). Obtained results show that all applied treatments significantly reduced the weed dry weight at 40 DAS and at harvest accompanied by significant increase in growth parameters of pea plants (plant height and dry weight of plant at 40 DAS), photosynthetic pigments, weight of pods/plant and seed yield/plant relative to unweeded treatment. It is obvious that herbicide at recommended dose was more effective than herbicide at ½ recommended dose. Meanwhile, treatment of plants with nano-salicylic acid significantly promoted pea plant growth and yield, especially at 100 ppm.

1. Introduction

Green peas are a popular vegetable. They are also quite nutritious and contain a fair amount of fiber and antioxidants. Additionally, research shows they may help protect against some chronic illnesses, such as heart disease and cancer. They have been part of the human diet for hundreds of years and are consumed all over the world. Green peas are one of the best plant-based sources of protein, which is a major reason why they are so filling, along with their high amount of fiber. Green peas contain a decent amount of heart-healthy minerals, such as magnesium, potassium and calcium. They may also have a positive effect on heart health. The high fiber content of green peas and legumes has been shown to lower total cholesterol and “bad” LDL cholesterol, both of which increase the risk of heart disease when they are elevated. Green peas also provide flavonols, carotenoids and vitamin C, antioxidants that have been shown to reduce the likelihood of heart disease and stroke due to their ability to prevent damage to cells. Eating green peas regularly may reduce the risk of cancer, mostly due to peas’ antioxidant content and their ability to reduce inflammation in the body. Green peas also contain saponins, plant compounds known for having anti-cancer effects. Several studies have shown saponins may help prevent several types of cancer and have the potential to inhibit tumor growth, several properties that may help prevent and treat some chronic diseases, such as heart disease, cancer and diabetes [1].

Bentazon is an herbicide approved for use in the EU. It is highly soluble in water, volatile and, as it is mobile, may present a risk of leaching to groundwater. Bentazon acts as a selective contact post-

emergence herbicide which is absorbed through the leaves of target plants and disrupts the photosynthetic process and causes a depletion of the carbohydrate reserves as well as disruption to the integrity of the chloroplast membranes. It is not likely to be persistent in soil systems but may be persistent in water under certain conditions. It is moderately toxic to humans and a recognized skin and eye irritant. Bentazon is also moderately toxic to birds, fish, aquatic invertebrates and earthworms [2].

The potential of nanotechnology for the development of sustainable agriculture has been promising. The initiatives to meet the rising food needs of the rapidly growing world population are mainly powered by sustainable agriculture. Nanoparticles are used in agriculture due to their distinct physicochemical characteristics [3, 4]. Salicylic acid (SA) is a phenolic compound and naturally occurring plant growth regulator that influences various physiological and biochemical functions in plants [5]. It can play a role as a regulatory signal mediating the response of plants to biotic and abiotic stresses. Furthermore, SA can regulate different physiological or developmental processes such as seed germination, vegetative growth, photosynthesis, respiration, thermogenesis, flower formation, seed production and senescence [6, 7].

The main aim of our work is to detect and compare the effects of different concentrations of the photosynthesis inhibitor bentazon on the activity of photosynthesis, the application of nano-salicylic acid and their interaction to control the negative effects of weeds on pea plants, in the addition to the question whether bentazon application in lower concentration could provoke defense responses in the non-target peas plants and cell death in the target weed plants.

2. Material and Methods

Two pot experiments were conducted at the green-house of the National Research Centre, Dokki, Cairo, Egypt on 5th of December during two winter seasons (2019/2020) and (2020/2021). Seeds of pea plants (*Pisum sativum*) were obtained from the Legumes Crops Research Department, Ministry of Agriculture and Land Reclamation, Egypt. Six pea seeds and *Anagalis arvensis* weed seeds (0.01 g) were sown in each pot (30 cm diameter) at a depth of 30 mm, in approx. 7 kg of clay: sand (3:1 v/v) soil. Granular ammonium sulphate (20.5 (w/w) % N) was applied at a rate of 40 kg N ha⁻¹, and single superphosphate (15 % P₂O₅) was added at a rate of 60 kg P₂O₅ha⁻¹ to each pot. These doses of nitrogen and phosphorous were added and mixed thoroughly into the soil of each pot immediately before sowing.



Photo : pea plants (*Pisum sativum*)

Ten days after sowing (DAS), pea seedlings were thinned leaving four uniform seedlings per pot. At 15 days old plants, pea seedling and its associated weeds were sprayed with bentazon herbicide at recommended dose (1 L fed⁻¹) and ½ recommended doses as well as by nano-salicylic acid at 50 and 100 ppm. Additionally, two control treatments, weed free and unweeded, were applied for comparison. Treatments were arranged in a complete randomized block design with six replicates for each treatment.

2.1. Data recorded:

2.2.1. Weeds

Three replicates were collected from each treatment at 40 days from sowing and at harvest. Weeds were dried in an oven at 40°C for 48 h to record dry biomass (g pot⁻¹).

2.1.2. Vegetative growth parameters of pea plants

Plants were collected from each treatment during vegetative growth stage (40 days after sowing) for measurement of growth parameters (plant height (cm), number. of leaves/plant, fresh and dry weights of plants).

2.1.3. Yield and yield attributes

At harvest, three replicates of pea plants were taken from each treatment to determine weight of pods/plant and seeds weight/plant.

2.1.4. Determination of photosynthetic pigments

Chlorophyll a, b and carotenoids (mg/g) were extracted from fresh leaves according to the procedure of Moran [8].

2.1.4. Determination of total carbohydrates, total soluble sugars and total polysaccharides

Total carbohydrates (CHO %), total soluble sugars (TSS %) and total polysaccharides (%) were determined according to the method described by Dubois *et al.* [9]. Total phenolic content was extracted from dry finally ground seeds according to the method described by Gonzalez *et al.* [10]

2.2. Statistical analysis:

All data were statistically analyzed according to Snedecor and Cochran [11] the treatment means were compared by using LSD at a 5% level of probability. Means were compared by using LSD at a 5% level of probability

Results and discussion

Data presented in **Table (1)** indicated that treatment of pea plants with the recommended dose of bentazon resulted in the death of all the weeds associated with pea plants. Similar results were also recorded in plants treated with recommended dose in addition to foliar treatment with 50 or 100 ppm nano-salicylic acid. On the other hand, application of ½ recommended dose of bentazon was less effective. Data presented in **Table (2)** indicated that treatment of pea plants with the recommended dose of bentazon in combination with the application of 100 ppm nano-salicylic acid resulted in the highest records of pea plant height, number of leaves/plant and fresh and dry weight/plant. Data presented in **Table (3)** indicated that application of the recommended dose of bentazon in combination with 100 ppm nano-salicylic acid significantly increased yield and yield components of pea plants, followed by plants treated with ½ dose of bentazon + 100 mM nano-salicylic acid in comparison with unweeded plants.

Table (1): Effect of the herbicide bentazon in combination with nano-salicylic acid on weed growth associated pea plants

Treatments	Concentration	40 Days After Sowing		The End Of The Season
		Weed FW	Weed DW	Dry weight
Weed free plants	0	0.00	0.000	0.00
Unweeded plants	0	74.85	9.485	27.00
Rec	1L/fed.	0.00	0.000	0.00
½ rec	½ L/fed.	9.30	3.096	2.04
S1	50mM	60.90	7.575	7.60
S2	100mM	45.10	5.07	5.60
Rec+S1	1L/Fed.+50mM	0.00	0.000	0.00
Rec+S2	1L/Fed.+100mM	0.00	0.000	0.00
½ Rec+S1	½ L/Fed.+50mM	14.35	1.542	4.51
½ Rec+S2	½ L/Fed.+100mM	9.55	1.314	2.90
LSD at 5%		1.96	0.101	0.51

S = nano-salicylic acid Rec = recommended dose

Table (2): Effect of the herbicide bentazon in combination with nano-salicylic on pea growth 40 days after sowing

Treatments`	Concentration	Plant height (cm)	No. of leaves/plant	Fresh weight (g/plant)	Dry weight (g/plant)
Weed free plants	0	46.66	14.66	5.13	0.660
Unweeded plants	0	32.50	10.66	2.40	0.423
Rec	1L/Fed.	47.00	14.83	5.10	0.876
½ Rec	½ L/Fed.	41.00	13.00	4.22	0.500
S1	50ppm	39.16	14.33	4.78	0.800
S2	100ppm	42.00	14.66	5.07	0.983
Rec+S1	1L/Fed.+50ppm	42.33	15.00	6.78	1.01
Rec+S2	1L/Fed.+100ppm	52.00	16.00	9.70	1.400
½ Rec+S1	½ L/Fed.+50ppm	44.33	14.16	4.90	0.500
½ Rec+S2	½ L/Fed.+100ppm	50.00	14.66	4.93	0.846
LSD at 5%		2.26	0.58	0.60	0.101

S = nano-salicylic acid Rec = recommended dose

Data presented in **Table (4)** indicated that all treatments significantly increased photosynthetic pigments in comparison with unweeded plants. Application of 1L/Fed bentazon +100 ppm nano-salicylic acid resulted in the highest chlorophyll a, chlorophyll b, carotenoids and total pigments contents in pea leaves after 40 days from sowing. Data presented in **Table (5)** indicated that treatment of pea plants with the herbicide bentazon, especially at ½ L/Fed significantly increased total phenols % in the seeds. Similar results were recognized for total carbohydrates % and total polysaccharides. Meanwhile, the highest total soluble sugars (TSS%) was obtained in unweeded plants followed by plants treated with ½ recommended dose of bentazon(½ L/Fed) + 100 ppm nano-salicylic acid. The use of nanotechnology in agriculture has increased considerably in recent years, and numerous benefits of nanoparticle applications have been reported [12, 13]. Nanoparticles have unique physicochemical properties compared with bulky particles: their small size and ability to cross barriers (cell walls and plasma membranes) facilitate effective absorption, while their large specific

surface can result in a good level of interaction with intracellular structures [14]. Consequently, nanoparticles can be used to increase the supply of elements to plant shoots and foliage.

Table (3): Effect of the herbicide bentazon in combination with nano-salicylic acid on pea yield.

Treatments	Concentration	Wt. pods/plant	No. Seeds/pod	Wt. Seeds/plant	Wt. 100 Seeds
Weed free plants	0	2.920	3.66	2.910	22.88
Unweeded plants	0	0.696	1.66	0.400	15.05
Rec	1L/Fed.	2.980	3.33	2.360	22.39
½ Rec	½ L Fed.	1.306	2.66	1.876	17.66
S1	50ppm	0.950	2.00	0.556	15.50
S2	100ppm	1.306	2.33	0.940	17.88
Rec+S1	1L/Fed.+50ppm	3.966	3.00	2.506	24.29
Rec+S2	1L/Fed.+100ppm	5.300	4.00	3.746	28.16
½ Rec+S1	½ L/Fed.+50ppm	3.636	2.79	2.360	19.88
½ Rec+S2	½ L/Fed.+100ppm	3.566	3.66	2.480	21.12
LSD at 5%		0.161	0.62	0.194	2.07

S= nano-salicylic acis Rec = recommended dose

Table (4): Effect of the herbicide bentazon in combination with nano-salicylic acid on chlorophyll a, chlorophyll b, carotenoids and total chlorophyll in pea leaves at 40 days after sowing

Treatments	Concentration	Chl. A	Chl. b	Carotenoids	Total Pigments
Weed free plants	0	2.220	0.580	0.370	3.170
Unweeded plants	0	1.835	0.520	0.310	2.665
Rec	1L/Fed.	2.135	0.565	0.375	3.075
½ Rec	½ L Fed.	2.040	0.550	0.345	2.935
S1	50 ppm	2.030	0.535	0.340	2.905
S2	100 ppm	2.095	0.555	0.360	3.010
Rec+S1	1L/Fed.+50 ppm	2.150	0.560	0.370	3.080
Rec+S2	1L/Fed.+100 ppm	2.835	0.620	0.375	3.830
½ Rec+S1	½ L/Fed.+50 ppm	2.175	0.550	0.345	3.070
½ Rec+S2	½ L/Fed.+100 ppm	2.285	0.565	0.375	3.225
LSD at 5%		0.060	0.021	0.014	0.098

S = nano-salicylic acis Rec = recommended dose

Nanoparticles of salicylic acid are promising as an efficient nutrient source for plants that can be used to improve biomass production by enhancing metabolic activities, photo-catalytic activity and conversion of light energy [15,16]; and by increasing the activity of enzymatic antioxidants such

as superoxide dismutase (SOD), catalase, and peroxidase, as well as by protecting chloroplast membrane structure [17]. Nianiou-Obeidat *et al.* [18], also reported that the glutathione S-transferase (GST) plays a role in the detoxification of various xenobiotics. GST activity and glutathione (GSH) levels also display circadian- and light-dependent patterns with a maximum late in the light and early dark phase.

Table (5): Effect of the herbicide bentazon in combination with nano-salicylic acid on some chemical constituents in pea seeds.

Treatments	Concentration	Total Phenols %	Total carbohydrates (CHO) %	Total Soluble sugars (TSS) %	Polysaccharides %
Weed free plants	0	3.890	60.275	10.705	49.570
Unweeded plants	0	5.940	41.600	14.550	27.050
Rec	1L/Fed.	6.265	62.590	11.775	50.815
½ Rec	½ L Fed.	7.025	65.020	12.125	52.895
S1	50 mM	5.565	55.745	12.300	43.445
S2	100 mM	4.010	57.590	11.735	45.855
Rec+S1	1L/Fed.+50 mM	5.700	61.850	11.210	50.640
Rec+S2	1L/Fed.+100 mM	5.495	60.600	12.250	48.350
½ Rec+S1	½ L/Fed.+50 mM	5.865	59.670	12.595	47.075
½ Rec+S2	½ L/Fed.+100 mM	5.845	61.520	13.035	48.485
LSD at 5%		0.287	0.588	0.412	0.733

S = nano-salicylic acid Rec = recommended dose

Salicylic acid (SA) is a phenolic compound and naturally occurring plant growth regulator that influences various physiological and biochemical functions in plants [5]. It can play a role as a regulatory signal mediating the response of plants to biotic and abiotic stresses. Furthermore, SA can regulate different physiological or developmental processes such as seed germination, vegetative growth, photosynthesis, respiration, thermogenesis, flower formation, seed production and senescence [5,6]. Bentazon acts as a selective contact post-emergence herbicide. It is absorbed through the leaves of target weed plants and negatively affects the photosynthetic process causing a depletion of the carbohydrate reserves. Bentazon also cause disruption to the integrity of the chloroplast membranes. As a result of its effect on photosynthesis and carbohydrate metabolism, bentazon results in weeds starvation and death [2].

Pospíšil [19] reported that bentazon inhibited chloroplastic electron transport (within a millisecond time range) causing a rapid and high production of reactive oxygen species (ROS). Biphasic ROS burst was described in the plant cell death process, where the first maximum of ROS was measured within minutes after the initial stimulus and the second, more intense burst occurred several hours later. The first ROS burst can originate from energy-organelle sources and both ROS maxima can be dependent on the basic antioxidant capacity and the activation of antioxidant enzymes [20]. ROS production and oxidative stress resulting in the rapid damage of lipids, carbohydrates, proteins and nucleic acids thus can inhibit active plant defense and induced cell death [21, 22]. Several researchers studied the effect of the activated antioxidant defense system. They found that it can substantially and rapidly contribute to cell survival by alleviating the primary and secondary oxidative stress effects of herbicides besides activating other xenobiotic detoxification pathways in plants. This was confirmed in both weeds and plants, where antioxidant enzymes were significantly

activated after xenobiotic exposure such as oxyfluorfen [23], linuron, dimethenamid [24], glyphosate [25] and paraquat [26]. These results verified the presence of oxidative stress and the significance of antioxidants in defense reactions in the cells of both sensitive and tolerant plants.

Bentazon is known as a photosynthetic inhibitor and the activity of antioxidant enzymes is highly dependent on the circadian clock and the light or dark conditions [27]. It was earlier observed that the activity of SOD, CAT and APX, as well as the content of APX substrate ascorbate (AsA) rose during the light phase of the day and lowered during the night in various plant species. The highest activity at the end of the light phase can be twice as much as at dawn [28-32].

Conclusion

The results suggested availability of using the antioxidant nano-salicylic acid with betazon herbicide to decrease the deleterious herbicide effect on growth and yield of *Pisum sativum* plants without affecting the selectivity and the efficacy of the herbicide on weeds and at the same time decreased weed propagation with significant degree.

Disclosure statement:

Conflict of Interest: The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards:

This article does not contain any studies involving human or animal subjects.

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