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Effect of seed priming on morphological characters with melatonin and nematicide in tomato (*Solanum lycopersicum* L.)

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Abstract
The investigation on effect of seed priming treatment on crop morphological parameters with melatonin and nematicide in tomato seed. Effect of melatonin and nematicide (Fluopyrum 34.48% SC) on morphological characters in 10 days old tomato seedlings were studied out at Department of Biochemistry, B.A. College of Agriculture, AAU, Anand. The experiment was carried out in laboratory with one cultivar *viz*. GAT-5 and different concentration of melatonin and nematicide soaked seed treatment. For selection of melatonin and nematicide concentration the morphological characters, such as mean germination time, germination index, root weight, root length, shoot weight and shoot length were measured. Significantly the best treatment of melatonin and Nematicide the seed priming treatment T_4 (50 µm Melatonin) and T_4 (1% Fluopyrum 34.48% SC), respectively.

Keywords: Priming treatments, tomato, growth parameters, crop growth, melatonin, nematicide (Fluopyrum 34.48% SC)

Introduction
Tomato (*Solanum lycopersicon* L., 2n=2x=24) belongs to the genus *Solanum* under the *Solanaceae* family. Tomato is the world's largest vegetable crop after potato and sweet potato, but it tops the list of canned vegetables. In world, tomato is second most widely cultivated vegetable crop after potato. China stands first in the major tomato growing countries followed by India, Turkey, Egypt, Iran, USA, Mexico, Italy, Brazil and Spain (Anonymous, 2020) [4].
Tomato is a power house of nutrition because of their high contents of essential nutrients and antioxidant-rich phytochemicals (Splittstoesser, 1990, Lorenz and Maynard, 1997, Singh *et al*., 2010) [23, 16, 22]. It has calorific value ranging from 20 to 40 calories per 100 g. The chemical composition varies depending upon variety and stage of maturity of the fruit. The ripe fruit contains around 94.5 percent water, 3.9 percent carbohydrates, 1.0 percent protein, 0.1 percent fat, 0.1 percent mineral matter, 0.01 percent calcium and 0.02 percent phosphorus. It contains important components which are essential for human in their diet like potassium, phosphorous, magnesium and iron as well as antioxidants such as carotenoids, lycopene and phenolics. It also contains vitamin A, ascorbic acid, vitamins B1, B6 and vitamin E (Giovanelli and Paradise, 2002 Odriozola-Serrano *et al*., 2008) [12, 18].
A tomato is one of the most important "protective foods" because of its special nutritive value due to higher content of lycopene. Consumption of lycopene has been reported to protect against cancer, cardiovascular diseases, cognitive function and osteoporosis. Lycopene having also anti-inflammatory, antidiabetic, anti-allergenic, anti-atherogenic, antithrombotic, antimicrobial, antioxidant, vasodilator and cardioprotective effects. Other carotenoids present in ripe tomato fruits include β-carotene and small amounts of phytoene, phytofluene, γcarotene, neurosporene and lutein. Many of the compounds present in tomato like quercetin, kaempferol, naringenin, *etc*., have antioxidant activities and are effective in protecting the human body against various oxidative stress- related diseases (Ali *et al.*, 2020) [2].
Seed priming is basically a pre-sowing-controlled seed hydration treatment in which seeds are soaked in an osmotic solution or solid carrier with low matrix potential that allows protrusion through the seed coat. The seed priming techniques improve the germination rate, uniformity of germination and sometimes greater total germination percentage (Basra *et al.*, 2006) [8].
Melatonin is synthesized in plants through a route from tryptophan. Melatonin may act directly as free radical scavenger on ROS/RNS, lipid peroxides, and toxic chemicals, controlling relevant aspects such as membrane integrity and the proper functioning of redox network.

Thus, melatonin acts as a first barrier against the ROS burst and, in a second line of defense, changes the expression of many responsive stress genes (Marino B. Arnao and Josefa Hernandez-Ruiz, 2015) [17]. Many studies (Dubbels *et al*., 1995 and Hattori *et al*., 1995) [11, 13] have clearly demonstrated that melatonin is present in different parts of plant such as seed, root, stem, leaf, flower and fruit. It activates the generation of root primordia and their subsequent growth into lateral roots and adventitious roots in *lupines albus*. Melatonin also could mitigate the oxidative stress, which may be induced by stress in tomato seedlings through scavenging of excess reactive oxygen species and increasing the stability of the cellular membranes (Jahan *et al.*, 2020) [14].

Tomato (*Solanum Lycopersicon* L.) is affected by various disease caused mainly by fungi, bacteria and nematodes. Nematodes found to be very fatal infective agents and cause severe yield loses. Root-knot nematodes (*Meloidogyne* spp.) are phytopathogenic obligate endoparasites nematodes that infect many plant species and cause serious damage to agricultural crops per year (Abad *et al*., 2008) [1]. Root-knot nematodes (*Meloidogyne* species), one of the most important groups of plant parasitic nematodes, have an exceedingly wide host range and interact with other plant pathogens.

Root knot nematodes are prevalent pests for many commercial crops. Their impact is felt in the loss of agricultural production and acute shortage of agricultural produce at a regional level. Oneof the most important vegetable crops in India that is most impacted by this pest is the tomato (*Lycopersicon esculentus*). The primary nematode species infesting tomato plants is the root knot nematode *Meloidogyne incognita*. The annual estimated crop losses due to major plant parasitic nematodes in India have been worked out to be about Rs. 242.1 billion.

The new strategies for the control of plant-parasitic nematodes have actively been sought during the last few years, and investigation has been focused more on biological control, organic and inorganic amendments, naturally occurring nematicides and induced resistance (Oka *et al*., 2000a) [19]. Management of plant parasitic nematodes has always been difficult, and the most successful strategy for many years has been the use of toxic fumigant nematicides, such as the most known methyl bromide (Oka *et al*., 2000b) [20]. The Nematicide Velum prime (Fluopyrum 34.48% SC). Velum prime is a revolutionary Nematicide that offers long lasting protection against Root Knot nematodes. Nematodes attack the roots of host crop and multiply very fast in favorable conditions resulting into formation of big knots on the roots. Amongst all nematodes seen in India, Root knot nematode is most prominently seen and causes substantial economic losses to the farmers. The active ingredient fluopyrum selectively inhibits Complex II of the mitochondrial respiratory chain of nematodes. Following application of Velum prime, nematodes cannot generate energy to sustain hence initially take on the shape of a needle, becomes immobile and eventually dies. Dosage - 250 to 300 ml/acre and must be applied up to 15 days of transplanting.

Materials and Methods

A laboratory experiment was carried out to study the Effect of seed priming on morphological characters with melatonin and nematicide in tomato (*Solanum Lycopersicon* L.) at Department of Biochemistry, B. A. College of Agriculture, AAU, Anand during the year 2020-21. One tomato variety (GAT-5) were kindly provided by Main Vegetable Research Station, AAU, Anand. For selection of various concentration of Melatonin and Nematicide for beneficial effect to seed germination of Tomato. (Seed priming for six hours.) GAT-5 were treated with melatonin and nematicide, eight different priming treatments including, T_1: Control, T_2: Water soak, T_3: 25 µM Melatonin, T_4: 50 µM Melatonin, T_5: 75 µM Melatonin, T_6: 100 µM Melatonin, T_7: 125 µM Melatonin, T_8: 150 µM Melatonin and T_1: Control, T_2: Water soak, T_3: 0.5% Nematicide (Fluopyrum 34.48% SC), T_4: 1.0% Nematicide (Fluopyrum 34.48% SC), T_5: 1.5% Nematicide (Fluopyrum 34.48% SC), T_6: 2.0% Nematicide (Fluopyrum 34.48% SC), T_7: 2.5% Nematicide (Fluopyrum 34.48% SC), T_8: 3.0% Nematicide (Fluopyrum 34.48% SC), respectively. All the primed seeds were used for laboratory conditions. A Completely Randomized Design (CRD) with three repetitions was adopted for the laboratory experiment.

Methodology for Priming

Seeds were subjected to priming with Melatonin (25 µM, 50 µM, 75 µM, 100 µM, 125 µM, 150 µM and nematicide (Fluopyrum 34.48% SC), 0.5%,1.0%, 1.5%, 2.0%, 2.5%, 3.0%, respectively for 6 hours. Seeds were soaked in priming solutions having double the volume of seed and ensured that seeds remained immersed in the solution, to avoid precocious germination during the treatment period. Seeds were dried back to the original moisture content under shade after 6 hours duration.

Observations Recorded

The observations on growth parameters in laboratory condition *viz*., Mean germination time (MGT), Germination index (GI), Germination (%), Root length (cm), Shoot length (cm), Root weight (mg), Shoot weight (mg), Seedling vigour index I (Length), were determined as per the ISTA Rules.

Statistical Methods

The observed data were statistically analysed by appropriate statistical procedures as suggested for completely randomized Design under laboratory conditions by Steel and Torrie (1980) [24].

Results and Discussion

Growth Parameters of Melatonin (Laboratory condition)

The effect of Melatonin on Growth characters of Tomato at 15 Days after germination

Germination%

The influence of melatonin on seed germination was tested and the data are presented in Table 1. The germination percent was varied between 86.76 to 100%. However, significantly maximum germination percent was observed in treatment T_4 (100%) which was followed by treatment T_2 (96.67%), T_3 (93.33%), T_5 (93.33%) and T_6 (93.33%). Seed priming treatment with melatonin by improving germination percent, as well as increasing speed and uniformity of germination. The higher dose of melatonin may reduce the germination percent. The reduction in germination might be due to degradation of the mitochondrial membrane leading to reduction in energy supply necessary for germination. Rosinska *et al.*, (2023) [21] have studied the effect of osmo priming with melatonin on germination, vigor and health of *Daucus carota* L. seeds and reported that melatonin enhanced the germination in carrot. They have also reported that osmo

priming with melatonin did not affect the uniformity of germination. Various scientists (Zhang *et al*., 2014, Xiao *et al.*, 2019; and Castanares and buzuo *et. al.*, 2019) [26, 25, 9] have also reported that melatonin enhanced the germination in cotton, cucumber and melon, respectively.

Mean germination time

Among the treatments, the significant difference was observed. However, significantly lower mean germination time was recorded in treatment T_4 (4.17), which was followed by T_5 (4.28), T_7 (4.41), T_6 (4.44), T_2 (4.52), T_8 (4.54), and T_3 (4.57). Significantly minimum mean germination time was observed for treatment T_1 (4.70). The lower mean germination time value means this treatment is best performance as compared to higher value of mean germination time. Thus, the treatment T_4 gave superior MGT as compared to all other treatments. It is a measure of the time it takes for the seed to germinate focusing on the day at which most seeds have germinated. Seed priming treatment with melatonin improved energy or speed of germination. Germination is the emergence and development of the seed embryo of those essential structures, which for the kind of seed being tested indicates the ability to develop into a normal plant under favorable conditions. Thus, the above result suggested that treatment T_4 is the best treatment to give higher mean germination time as compared to remaining treatments.

Germination index

The germination index reflects the percentage of germination on each day of the germination period. The significant difference was recorded germination index. Significantly the higher germination index was recorded for treatment T_4 (2.44) which was at par with treatment T_5 (2.41), T_6 (7.40), T_2 (2.29), T_1(2.28), T_3 (2.25). The non-significant and minimum germination index was recorded for treatment T_8 (2.18) and T_7 (2.21). Germination index indicated the quality of seed germination. The higher germination index means the quality of germination is superior as compared lower value germination index. Germination index is a character that combines the percentage and time of germination thus the faster a seed lot has germinated, the higher the germination index. The results observed here are in agreement with the result obtained by Jiang *et al.,* (2016) [15] who have studied the seed priming with melatonin effects on seed germination and seedling growth in maize under stress. They have suggested from their experiments that seed priming with 0.8 Mm melatonin significantly improved germination index. Thus, the above result suggested that treatment T_4(50 µM) is the best treatment to give higher germination index as compared to remaining treatments.

Root weight (g)

The effect of melatonin on root weight was recorded and data are presented in Table 1.1.(A). The maximum and minimum root weight was recorded for treatment T_4 (0.039 g) and T_8 (0.028 g), respectively. Thus, the below result suggested that treatment T_4 is the best treatment to give higher root weight as compared to remaining treatments The present finding with is in agreement with previous workers (Bajwa *et al*., 2014) [7], who reported that role of melatonin in alleviating stress in *Arabidopsis thaliana*. They have recorded that melatonin treated plant (10 and 30 µM) had significantly higher fresh root weight as compared with nontreated seed. Zhang *et. al.*, (2013) [27] have also concluded that melatonin enhanced cucumber root weight.

Root length (cm)

The root length was varied from 0.81 to 1.26 cm. The significantly maximum and minimum root length was observed for treatment T_2 (1.26 cm) and T_8 (0.81cm). However, the non-significant difference was found among treatments T_8 (0.81 cm), T_7 (0.86 cm), and T_3 (0.87 cm). Thus, the result suggested that treatment T_4 is the best treatment to give higher root length as compared to remaining treatments. The decline in seedling root length might be attributed to DNA degradation with ageing leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination. The results observed here are in agreement with the result obtained by various scientists (Arnao and Hernandez Ruiz., 2013; Bajwa *et al.,* 2014) [6, 7].

Shoot weight (g)

Among the treatments, significantly higher shoot weight was recorded for treatment T_4 (0.051 g), which was followed by T_5 (0.048 g), T_6 (0.047 g), T_3 (0.046 g), T_7 (0.045 g), T_2 (0.044 g), and T_8 (0.042 g). However, the significantly lower shoot weight was recorded in treatment T_1 (0.039 g). Thus, the result suggested that treatment T_4 is the superior over other treatments to give higher shoot weight as compared to remaining treatments The results observed here are in agreement with Altaf *et. al.*, (2022) [3] who reported that the application of melatonin in tomato seed improved significantly increase in shoot weight.

Shoot length (cm)

The significant difference was observed in shoot length (cm). Among the various treatments, significantly higher and lower shoot length was recorded for treatment T_2 (2.63 cm) and Treatment T_8 (2.36 cm), respectively. Thus, the result suggested that treatment T_4 is the best treatment to give higher root length as compared to remaining treatments. Similar results are also reported by Bajwa *et al.* (2014) [7] they have noted that melatonin treated plant (10 and 30 µM) had significantly higher fresh shoot length compared with non-treated seed in *Arabidopsis.*

Vigor index-I

Significantly the highest vigor Index was recorded for Treatment T_4 (7.67). The significantly lower vigor index was recorded for treatment T_8 (6.03), which was at par with the treatment T_1 (6.10) and Treatment T_7 (6.44). Thus, the above result suggested that treatment T_4 i.e.,50 µM seed priming treatment is given the best performance to give higher vigor index as compared to remaining treatments. High-vigor seeds indicate improve of seed germination, seedling emergence, increase crop yield and reduce the cost of agriculture production. It is also used as indicator of the storage potential of a seed lot and in ranking various seed lots with different qualities. Similar result is reported by Dawood (2018) [10] and Jiang *et al.*, (2016) [15] in faba bean and maize, respectively.

Table 1: The effect of various concentration of Melatonin for beneficial effect to Growth characters of 15 DAG old Tomato seedlings

	GI	MGT	Vigour index-i	Germinati on %	RW (mg)	RL (cm)	SW (mg)	SL (cm)
T_1	2.28	4.70	6.10	90.00	0.032	1.12	0.039	2.59
T_2	2.29	4.52	6.93	96.67	0.035	1.26	0.044	2.63
T_3	2.25	4.57	6.74	93.33	0.030	0.87	0.046	2.43
T_4	2.44	4.17	7.67	100.00	0.039	1.08	0.051	2.58
T_5	2.41	4.28	6.97	93.33	0.036	1.01	0.048	2.48
T_6	2.40	4.44	6.88	93.33	0.034	0.90	0.047	2.46
T_7	2.21	4.41	6.44	90.00	0.032	0.86	0.045	2.40
T_8	2.18	4.54	6.03	86.67	0.028	0.81	0.042	2.36
S. Em. ±	0.036	0.095	0.139	3.333	0.001	0.030	0.001	0.035
C.D at 5%	0.108	0.286	0.418	9.998	0.003	0.089	0.003	0.106
C.V.%	2.70	3.71	3.59	6.21	5.07	5.25	3.30	2.45

Treatments: T_1: Control, T_2: Water soak, T_3: 25 µM Melatonin, T_4: 50 µM Melatonin, T_5: 75 µM Melatonin, T_6: 100 µM Melatonin, T_7: 125 µM Melatonin, T_8: 150 µM Melatonin

The effect of Nematicide (Fluopyrum 34.48% SC) on Growth characters of Tomato at 15 Days after germination

Germination (%): The effect of Nematicide (Fluopyrum 34.48% SC) on germination was recorded and data are presented in Table 2. The significant difference was recorded among treatments However, the higher mean germination percent was observed in treatment T_4 (100%). The lower germination % was recorded in treatment T_8 (80.00%), which was at par with treatment T_7 (83.3%) and Treatment T_6 (83.3%).

Mean germination time: Non-significant difference was recorded in mean germination time (Table 2). However, the maximum and minimum germination time was observed for treatment T_1 (4.66) and T_4 (4.23).

Germination index: The germination index reflects the percentage of germination on each day of the germination period. Significantly the highest germination index was recorded for treatment T_4 (2.43). Significantly lower germination index was recorded for treatment T_8 (1.81), which was at par with treatment T_7 (1.83) and T_6 (1.85). Germination index indicated the quality of seed germination. The higher germination index means the quality of germination is superior as compared lower value germination index.

Root weight (g)

The root weight from different treatments of Nematicide imposed to tomato was tested and results are presented in Table. 2. Among the treatments, the significantly difference was observed. Significantly the highest root weight was recorded in treatment T_2 (0.0364 g). Nonsignificant and lower root weight was recorded for treatments T_8 (0.0283 g), T_3 (0.0294 g) and T_7 (0.0298 g)

Root length (cm)

The maximum and minimum root length was recorded for treatment T_2 (1.693 cm) and T_7 (1.407 cm). Significantly higher and non-significant difference of root length was recorded for treatment T_2 (1.693 cm), T_4 (1.638) and T_3 (1.605 cm).

Shoot weight (g)

The effect of Nematicide on shoot weight was analyzed and data are depicted in Table.

2. Among the treatments, significantly the maximum shoot weight was recorded for T_2 (0.053 g), which was at par with treatment T_4 (0.049 g). The minimum shoot weight was recorded for treatment T_8 (0.035 g), which was at par with treatment T_7 (0.039 g).

Shoot length (cm)

The significant difference was observed among all treatment for shoot length (cm). Among the various treatments, the higher shoot length was recorded for water soak treatment T_2 (3.01 cm), which was followed by treatments T_4 (2.97 cm), T_1 (2.91 cm) and T_5 (2.77 cm).

However, the significantly lower shoot length was recorded for treatment T_8 (2.52 cm).

Vigour index-I: The data presented in Table.2 indicated the beneficial effect of Nematicide on Vigour index. Among the treatments, the significant difference was observed higher vigor index in treatment T_2 (3.007), which was followed by T_4 (2.968) and T_1 (2.914). However, the significantly minimum vigor index was recorded for treatment T_8 (2.520).

Table 2: The effect of various concentration of Nematicide (Fluopyrum 34.48% SC) for beneficial effect to Growth characters of 15 DAG old Tomato seedlings

	MGI	MGT	Vigour Index-I	Germina Tion %	RW (mg)	RL (cm)	SW (mg)	SL (cm)
T_1	2.16	4.66	2.914	90.0	0.0342	1.545	0.048	2.91
T_2	2.09	4.32	3.007	93.3	0.0364	1.693	0.053	3.01
T_3	2.14	4.59	2.749	90.0	0.0294	1.605	0.046	2.75
T_4	2.43	4.23	2.968	100.0	0.0337	1.638	0.049	2.97
T_5	2.03	4.48	2.771	90.0	0.0314	1.567	0.047	2.77
T_6	1.85	4.44	2.554	83.3	0.0306	1.523	0.042	2.55
T_7	1.83	4.55	2.529	83.3	0.0298	1.407	0.039	2.53
T_8	1.81	4.58	2.520	80.0	0.0283	1.420	0.035	2.52
S.Em	0.026	0.102	0.038	2.041	0.001	0.035	0.001	0.038
C.D at 5%	0.077	NS	0.115	6.12	0.002	0.104	0.004	0.113
C.V%	2.19	3.94	2.42	3.98	3.76	3.88	4.71	2.38

Treatments: T_1: Control, T_2: Water soak, T_3: 0.5% Nematicide (Fluopyrum 34.48% SC), T_4: 1.0% Nematicide (Fluopyrum 34.48% SC), T_5: 1.5% Nematicide (Fluopyrum 34.48% SC), T_6: 2.0% Nematicide (Fluopyrum 34.48% SC), T_7: 2.5% Nematicide (Fluopyrum 34.48% SC), T_8: 3.0% Nematicide (Fluopyrum 34.48% SC)

Conclusion

Seed priming is known as quality enhancement of seed which give better result during germination and seedling growth. The experiment was carried out in laboratory with GAT-5 cultivar and eight seed priming treatments of different melatonin and nematicide (Fluopyrum 34.48% SC) concentration. The effect of melatonin on growth characters of tomato at 15 days after germination. The growth attributes such as germination time, germination index, root length, root weight, shoot length, shoot weight, germination percent and vigour index were observed higher with seed priming treatment T_4 (50 µM Melatonin). Thus, the exogenous melatonin could improve the germination characteristics of tomato seed with treatment T_4. The effect of nematicide (Fluopyrum 34.48% SC) on growth characters of tomato at 15 days after germination. The growth attributes such as germination time, germination index, root length, root weight, shoot length, shoot weight, germination percent and vigour index were observed higher with seed priming treatment T_4 (1% Fluopyrum). Thus, the exogenous nematicide could improve the germination characteristics of tomato seed with treatment T_4.

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