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EFFECTS OF MELATONIN ON ROOT KNOT NEMATODE: *IN SITU* ESTIMATION OF PHYSIOLOGICAL RESPONSES IN TOMATO

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Abstract

This research was conducted in 2020 to investigate the influence of melatonin (10, 50 and 100 µM) given in three methods (immersion, irrigation and foliar spraying), on some physiological aspects of tomato seedlings exposed to root knot nematodes. The seedlings were inoculated with 1000 infective juveniles of Meloidogyne incognita [(Kofoid and White) Chitwood]. Dualex® optic sensor was used to in situ measure total chlorophylls, flavonols and anthocyanins contents and nitrogen balance index (NBI). Results indicated that while no significant effects were observed on chlorophyll content, melatonin ameliorated the adverse effects of M. incognita on chlorophyll depending on the concentration and mode. Flavonols were at the highest in the irrigated plants and the lowest in the immersed ones, between 06-1.1, exhibiting a significant difference. NBI was affected by the method the melatonin was applied, and immersing boosted it, while irrigation caused a significant decrease. Irrigation resulted in significantly higher anthocyanins compared to the other two methods. One observation was that applying melatonin in the low and medium concentrations to the nematode containing soil increased the anthocyanins. Melatonin merits a value in developing a response against the nematode but needs further elucidation.

Key words*: anthocyanins, chlorophyll, flavonoids, Meloidogyne incognita, NBI.*

INTRODUCTION

Pathogen attacks are one of the growth- and quality-limiting factors plants face throughout their lives. Ameliorating or completely evading these stress factors rely on plant's innate resistance capacity as well as the favourable support from environment. Nematodes, especially root knot nematodes (RKN) *Meloidogyne* spp., are one of the major life-threatening pests that use hundreds of plants as hosts. Four predominant species of *Meloidogyne* species are *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*. Among the symptoms plants indicate after infection are chlorophyll loss in leaves, nutrient insufficiency resulting in growth loss and if persists, plant death. Due to damage in vascular tissues of root, water and nutrients can fail to move through the plant (Ralmi et al., 2016).

Despite that RKNs could be managed with the use of chemical nematicides, increasing crop production in an ecologically friendly manner is both a necessity and a challenge in a world with increasing awareness in human and environment health (Collange et al., 2011). In the recent years, melatonin, a naturally existing compound in plants, has attracted an interest in boosting sustainable crop production due to its beneficial effect on human health and environment (Reiter et al., 2015; Qiao et al., 2019). Melatonin functions as an antioxidant against reactive oxygen and nitrogen species (Moustafa-Farag et al., 2020).

Recent years in melatonin research have focused on its effects on plant growth and development along with its protective role against abiotic stress factors. Readers have an opportunity to follow some excellent reviews on the subject (Sharif et al., 2018; Yu et al., 2018). However, its effects on helping plants cope with biotic stress factors have not been explored in as much detail. Yin et al. (2013), Wei et al. (2017), Zhang S. et al. (2017), Aghdam et al. (2017) and Liu et al. (2019) have presented improving effects of melatonin against fungal pathogens in apple, banana, potato, strawberry, and tomato, respectively. It was reported that protection comes from

increase in defence gene expression, scavenging reactive oxygen species, raise in nitric oxide production and cell wall thickening (Shi et al., 2015; Wei et al., 2018; Zhao et al., 2019). However, to our best knowledge, there is not a study that involves influence of melatonin against root knot nematode attacks on plants.

Early assessment of stress symptoms is possible with non-destructive monitoring of crops using optical or thermal sensors. *In situ* and *de novo* changes in the physiological aspects of plant development can enable to have the results quickly and periodically with ease (Padilla et al., 2014). Among these sensors is Dualex® (Orsay, France), a leaf-clip chlorophyll-meter which estimates chlorophyll (CHL) and leaf epidermal flavonoids as well as anthocyanins using CHL fluorescence screening method (Agati et al., 2016). Non-destructive optical tools use spatial and temporal dimensions to assess adaptability levels of plants under stress conditions (Barnes et al., 2015). Dualex® have been used for many abiotic stress related studies for early detection of symptoms, for instance, heat (Zhou et al., 2017), chilling (Oustric et al., 2017) and UV (dos S. Nascimento et al., 2020). In more recent years, it has been also utilized for grapevine leaf stripe disease (Di Gennaro, 2016), root-knot nematodes in eggplant (Silva-Sánchez et al., 2019), reniform nematode in cotton (Singh et al., 2020) and wheat stripe rust (Emebiri et al., 2020).

This study was planned to assess the effects of melatonin given through immersion, irrigation, and spraying on some physiological characteristics measured with the Dualex® of tomato plants exposed to *M. incognita.*

MATERIALS AND METHODS

Seedlings of commercially processing tomato cultivar (H2274; *Lycopersicon esculentum* L.) with 3-4 established leaves were used as the plant material. Seedlings were separated in three groups according to the method the melatonin solutions were given: root-immersion, root-irrigation, and foliar spraying. Melatonin (Mel) obtained from Merck (M2250) was prepared in three concentrations (10, 50 and 100 µM). The trial also had one negative control (only distilled water), and one positive control (distilled water plus nematode inoculation). Mel was applied to the seedlings with and without nematode inoculation of 1000 infective juvenile stage 2 (IJ_2) per ml of *Meloidogyne incognita* reproduced from a single egg mass. Seedlings were planted in 1.4-liter plastic pots containing sterilized soil and sand mixture (approx. 450 g). Pots later were placed in a growth chamber under the conditions of 26 ± 1°C and 18/6 hours day/night light periods. Plants were only irrigated on the basis of need at fixed amount (50 ml).

Root-immersion: After the growth medium around the roots of the seedlings were cleared under tap water, the roots were tap dried with a paper towel and they were kept in the melatonin solutions for 10 minutes. Control groups were kept in the distilled water for the same duration.

Root-irrigation: The seedlings were irrigated with the melatonin solutions at an amount of 10 ml/pot. Control groups were irrigated with 10 ml distilled water. Second (20 ml/pot) and third (40 ml/pot) applications were done 1 week apart.

Foliar-spraying: After planting in the pots, the leaves of the seedlings were sprayed with 10 ml Mel solutions until the runoff. Control groups were sprayed with distilled water. Second (20 ml/pot) and third (40 ml/pot) applications were done 1 week apart.

Optical measurements

At the end of 8 weeks (on the 56th day), optical measurements with the Dualex Scientific+ Chlorophyll and Polyphenol-Meter (Force-A, Centre Universitaire Paris-Sud, France) were performed on the third youngest leaf. The portable meter Dualex® allowed simultaneous readings from the abaxial and adaxial surface of the leaves and provided a mean value for each reading. Three readings per leaf away from the midrib were made. Features measured were chlorophyll (µg per cm^2), relative absorbance units of flavonols (0 to 3) and anthocyanins (0 to 1.5), and the nitrogen balance index, determined by the relationship between chlorophyll and flavonols.

Statistical analysis

The experiment was arranged in a completely randomized design with three replications. The

data of chlorophyll, flavonols, anthocyanins and nitrogen balance index (NBI) were analyzed and corresponding graphs were plotted R version 4.0.2 (2020-06-22). There were 6 levels of Mel concentrations with and without nematode application, 2 control groups and 3 levels of mode. Every treatment consisted of three replicates of one plant per pot.

RESULTS AND DISCUSSIONS

Melatonin given in three modes (i.e., root-immersion, root-irrigation and foliar-spraying) resulted different responses in chlorophyll, flavanols, anthocyanins and nitrogen balance index in tomato plants inoculated with *Meloidogyne incognita.*

Although chlorophyll amounts were not affected by none of the treatments (Figure 1), generally immersed plants had more CHL than the irrigated or sprayed ones. Comparing with the negative controls showed that nematode addition caused 40-50% decrease in the positive control plants.

Treating plants with Mel resulted in as much CHL, depending on the concentration, as in the DW treated ones. It was observed that Mel in the nematode inoculated plants ameliorated the adverse effects on CHL depending on the concentration and type of administration, for instance immersing in 100 µM Mel or spraying with 50 µM Mel, respectively, provided increased levels of CHL.

It is believed that primary site for melatonin production is chloroplasts (Martinez et al., 2018) and Weeda et al. (2014) stated that melatonin can act as a protectant of CHL content. Wang et al. (2013) also reported decreased levels of chlorophyll-degrading enzyme, pheide-a-oxygenase with melatonin. Zhang et al. (2014) expressed that melatonin, when applied exogenously, protected CHL in the apple leaves exposed to abiotic stress. *Malus hupehensis* Rehd seedlings had les chlorophyll degradation when root-treated with melatonin (Tan et al., 2007a). Yin et al. (2013) showed that apple plants irrigated with melatonin had comparably close contents of chlorophyll to the control plants when infected with Marssonina apple blotch. Sun et al. (2019) indicated that melatonin treated cucumber against downy mildew had increased levels of chlorophyll. Similar activity was observed in this research where tomato plants exposed to nematode damage.

Plants respond to stress factors by synthesizing flavonols (Brunetti et al., 2013). R analysis indicated that the mode had a significant influence on flavonols in the tomato plants (Figure 2a). They were the highest in the irrigated plants, ranging from 0.9-1.5 and the lowest in the immersed ones, between 06-1.1. Flavonoids are formed when plants are exposed to pests (Brunetti et al., 2013). Bali et al (2018) stated that flavonoid contents raised in the seedlings of tomato plants treated with jasmonic acid against root knot nematode. Although no significant effects of melatonin were detected in the present study, how it is received by the intact plants showed a clear importance. One possible reason for spraying to have had the lowest amount of flavonols might be the anatomical structure of the leaves (i.e. the trichomes and undulation on the surface), owing to the studies have shown that flavonoids are placed in epidermal layers or in the cuticle of leaves (Tattini et al., 2004), which might have been a barrier for the solution to infuse,

In the current study, NBI level was affected by the method melatonin was applied to the plants, and immersing boosted it while irrigation caused a significant decrease (Figure 2b). It is considered a general acceptance that plant nitrogen status of a plants can be estimated through chlorophyll and flavonoid contents (Agati et al., 2016). Because chlorophyll is incorporated in chlorophyll (Evans et al., 2001), and flavonoids contents act oppositely to N contents in the plant, the ratio of CHL to flavonols is shown to be a more sensitive indication of plant nitrogen status (Longchamps and Khosla, 2014; Padilla et al., 2014). Kautz et al. (2014) indicated that under saline conditions the tomato leaves had elevated levels of NBI. Sun et al. (2019) reported increased levels of enzymes in N metabolism in cucumber seedlings treated with melatonin against *Pseudoperonospora cubensis*. The plots of both CHL and NBI show that root-immersion and spraying were better at abating the adverse effects of *M. incognita*. Scientific evidence indicates that growth and

development in plants are closely regulated by auxin and melatonin sharing a precursor with auxin might aid same processes (Arnao and Hernandez-Ruiz, 2007). Direct contact with

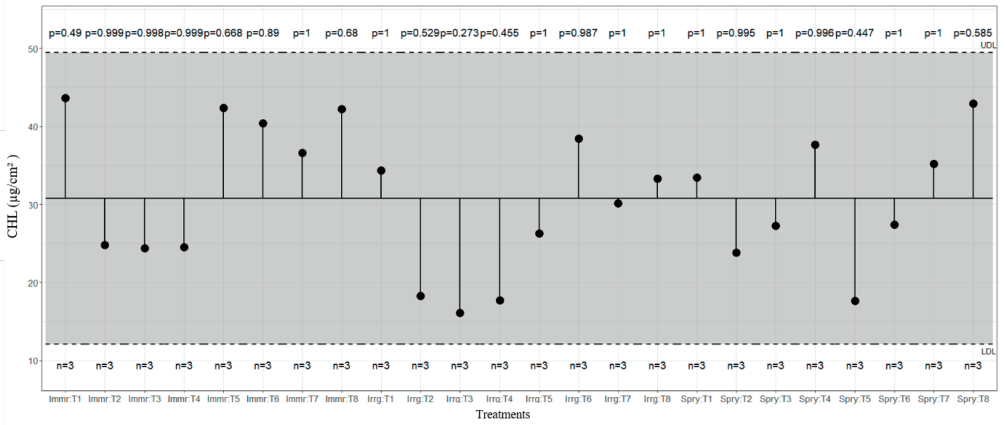
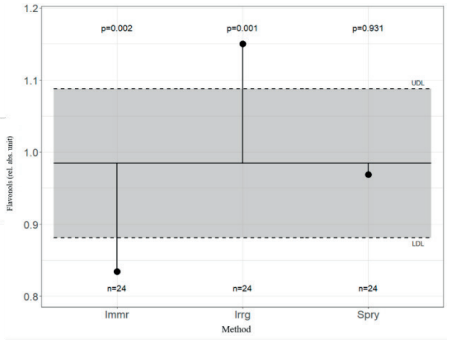
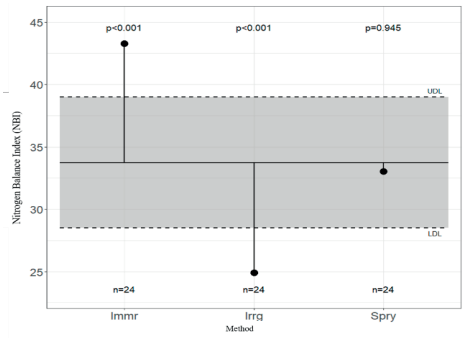


Figure 1. Effects of melatonin given in three methods to the tomato plants exposed to *Meloidogyne incognita* on chlorophyll content. Abbreviations; imm: immersion, irr: irrigation, spr: foliar spraying, T1: distilled water, T2: distilled water+ nematode, T3: 10 µM mel + nematode, T4 50 µM mel + nematode, T5: 100 µM mel + nematode, T6: 10 µM mel, T7: 50 µM mel, T8: 100 µM mel

melatonin in the roots through immersion for 10 min. might have induce level of auxin and improved root activity (Chen et al., 2009) and efficiency in nitrogen uptake and metabolism (Zhang R. et al., 2017).

Anthocyanins were affected both by the treatments and the methods they were given to the plants (Figure 2c, d). Irrigation resulted in significantly higher anthocyanins compared to the other two (Figure 2c). Plants having nematode but no melatonin (T2) produced the highest amounts of anthocyanins (Figure 2d). The rest of the treatments fell in the same group showing similar values. One observation was that applying Mel in the low and medium concentrations to the nematode containing soil increased the anthocyanins. On the other hand, Mel alone seemed not supportive of the anthocyanin production. Although response of anthocyanin accumulation under stress conditions differed in plants, melatonin appeared to have an increasing effect on nematode-inoculated tomato in the current study. Similar observation was confirmed by Zhang et al. (2016) on cabbage seeds. Bali et al. (2018) reported increased levels of anthocyanins in the tomato plants after jasmonic acid application, indicating that signalling molecules have a stimulating effect on antioxidative defence system.





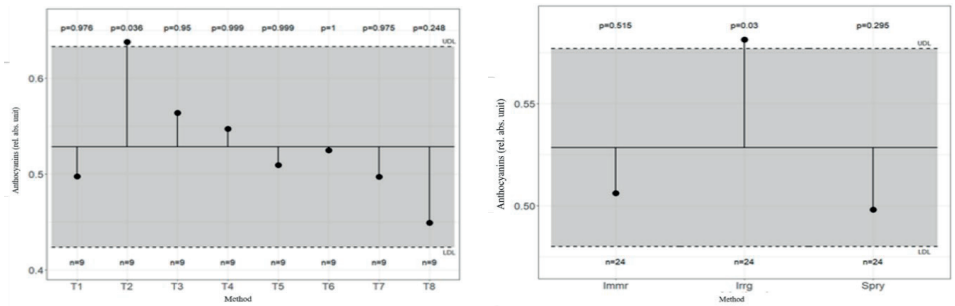


Figure 2. Effects of melatonin given in three methods to the tomato plants exposed to *Meloidogyne incognita* on flavonols (a), NBI (b), and anthocyanins (c and d). Abbreviations; imm: immersion, irr: irrigation, spr: foliar spraying, T1: distilled water, T2: distilled water+ nematode, T3: 10 µM mel + nematode, T4 50 µM mel + nematode, T5: 100 µM mel + nematode, T6: 10 µM mel, T7: 50 µM mel, T8: 100 µM mel

CONCLUSIONS

Root knot nematodes have been a subject of plant survival studies due to their countless number of hosts and above ground symptoms costing a loss in yield and in extreme cases, plant's life. Using synthetic chemical-based compounds for protection has raised environmental and public health safety, therefore a tendency to utilize more friendly approaches increases. Melatonin, being a safe molecule both present in humans and plants, is becoming a centre of studies for its regulatory and supporting roles in plant's growth and development. Results of this study indicates that it merits a value in developing a response against the nematode but needs further elucidation. This response might come from protection of chlorophyll content, therefore preserving nitrogen balance in the plant, as well as stimulating defence mechanisms. Different concentrations with varying exposure time are needed to elucidate these responses.

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