Evaluation of Allelopathic Effects of *Nigella sativa* L. against Rigid Ryegrass

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BulletinUASVM Horticulture 76(1) / 2019

Print ISSN 1843-5254, Electronic ISSN 1843-5394

DOI:10.15835/buasvmcn-hort: 2018.0045

Abstract

A pot experiment was conducted to evaluate the allelopathic potential of *Nigella sativa* L. on seedling emergence and first growth of the serious weed rigid ryegrass (*Lolium rigidum* L.). The results revealed significant weed suppressive effects of *N. sativa* residues incorporated into the soil. The lowest seedling emergence rate (38%) was observed in pots treated with 20 g leaf residues. Regarding height, the lowest value (26.7 cm) was found in 20 g root residues. The highest quantity of shoot residues reduced leaf number, tiller number, fresh and dry aerial biomass up to 58, 51, 64 and 59%, respectively. It can be concluded that the effect of incorporation of plant residues depended on the type and amount of residue. *N. sativa* could be utilized as an allelopathic crop to control weeds and future studies should focus on isolating and identifying allelochemicals in roots, shoots and leaves of this species.

Keywords: Allelopathy, integrated weed management, *Lolium rigidum*, *Nigella sativa*, plant growth.

Introduction

The widespread evolution of herbicide resistant weed biotypes and the increasing environmental concern about the impact of synthetic herbicides require the exploring of new possibilities for weed control. Allelopathy can provide an alternative to promising weed control methods, since it is considered as the plants' ability to produce chemical compounds, called allelochemicals, which are released into the environment and can have a direct or indirect harmful or beneficial effect on the growth, survival, development and reproduction of other plants or even microorganisms (Rice, 1984; Cruz-Silva *et al.*, 2016). Allelochemicals are secondary plant metabolites, including terpenoids, phenolic compounds, long chain fatty acids, organic cyanides, alkaloids, and others, which are synthesized by the plants during their growth and

developmental period, and can affect physiological functions like respiration, photosynthesis and ion uptake (Macías *et al.*, 2006). During the last decades, the use of plant species with strong allelopathic properties has shown promising prospect for natural herbicides development (Travlos *et al.*, 2008; Bilalis *et al.*, 2013).

Nigella sativa L. (Family: *Ranunculaceae*) is an annual herbaceous plant, widely growing in many parts of the world and particularly the Mediterranean region, North Africa, Middle East and some parts of Asia. It is a commercial medicinal plant since its seeds are traditionally used as a medicament for a variety of disorders in the respiratory system, cardiovascular system, digestive tract, kidney, liver, and immune system (Paarakh, 2010). In addition, a number of studies have been reported that this species presents a

strong allelopathic potential (Fawzia *et al.*, 2007; Zribi *et al.*, 2014).

Rigid ryegrass (*Lolium rigidum* Gaud.) consists one of the most important grass weeds in Mediterranean climates, because of its wide diversity, abundance and harmful effects on winter cereals and other crops (Steadman *et al.* 2004). It also displays multiple developed resistance across several herbicide chemistries/modes of action due to the high levels of genetic diversity (Tranel and Trucco, 2009). Considering that very few studies have assessed the allelopathic effects of *N. sativa*, the purpose of the present study was to evaluate the allelopathic ability of *N. sativa* residues on the seedling emergence and first growth of the serious weed rigid ryegrass.

Materials and Methods

Plant material was collected at the reproductive stage of *N. sativa* crop established in the experimental field of Agricultural University of Athens (37° 59' 01.5" N, 23° 42' 07.2" E) in June 2016. For the purpose of the present allelopathic study, leaves, shoots and roots of *N. sativa* plants were used. The selected material was air-dried and then enclosed in air-tight plastic bags until further use.

A pot experiment was conducted in a greenhouse of the Agricultural University of Athens (37° 59' 01.7" N, 23° 42' 10.6" E) from February to April 2017 in order to investigate the inhibitory efficacy of each of above-mentioned *N. sativa* tissues on seedling emergence and first growth of rigid ryegrass. The experiment was carried out according to a completely randomized design (CRD), with seven treatments and three replications for each treatment. Specifically, the treatments were as follows: untreated (control), 10 and 20 g of root residues, 10 and 20 g of shoot residues, 10 and 20 g of leaf residues. On the 2nd of February 2017, the plant residues were incorporated into the soil and after 20 days, rigid ryegrass seeds, characterized by high germinability, were uniformly dispersed (40 seeds per pot) in 9.5 L pots (24 cm diameter) containing only soil and watered as required. The soil was a clay loam (34.1% clay, 28.8% silt and 37.1% sand) with pH (1:1 H₂O) 7.29, 1.47% organic matter (Walkley and Black, 1934), 13.4% nitrogen content, maintaining adequate levels of nitrate (104.3 ppm), moderate in available phosphorus

(9.95 ppm) and high in available potassium (590 ppm) and sodium (110 ppm).

The rate of seedling emergence was estimated at 11 and 19 days after sowing (DAS). The growth parameters measured in this study were plant height (11, 19, 27, 34, 40, 52 DAS), number of tillers, number of leaves per plant (27, 34, 40, 52 DAS), as well as fresh and dry weight of above-ground parts (40, 52 DAS), and determined using three randomly selected plants from each pot. The dry weight was assessed after drying for 48 hours at 62°C (Travlos *et al.*, 2018).

The experimental data were analyzed using the JMP 8 statistical software (SAS Institute Inc., Cary, USA) according to the completely randomized design (CRD). Values were compared by analysis of variance (ANOVA) and differences between means were separated using the Least Significant Difference (LSD) test. All comparisons were made at the 5% level of significance ($p \leq 0.05$).

Results and Discussions

The rate of seedling emergence was significantly affected by the different plant tissues (Fig. 1). The lowest rates (16.86% and 38.43% at 11 and 19 DAS, respectively), were found in pots treated with 20g of *N. sativa* leaf residues (Tab. 1). According to a previous study, the aerial parts of *N. sativa* presented higher content of total phenolics, flavonoids, flavonols and flavones, and proanthocyanidins as compared to its seeds, and their aqueous extracts reduced germination rate and affected seedling growth of lettuce (Zribi *et al.*, 2014). In addition, Zribi *et al.* (2018) demonstrated that the organic extracts of *N. sativa* aerial parts presented also inhibitory effect on the lettuce germination. Specifically, the chloroform extract of aerial parts harvested at fruiting stage reduced the final germination by 50%.

The results obtained from the combined analysis of variance revealed that the height of rigid ryegrass was significantly influenced by the different residues (Tab. 1). Significant differences were found at 19 and 40 DAS, where the lowest values (7.38 and 26.74 cm at 19 and 40 DAS, respectively) observed in rigid ryegrass plants treated with the double quantity (20 g) of *N. sativa* root residues. It is worth noting that the high quantity of leaf residues also presented remarkable effect on plant height of rigid ryegrass at 19 and 40 DAS, as rigid ryegrass plants obtained the second

Figure 1. Effect of *Nigella sativa* plant residues on seedling emergence of rigid ryegrass. Vertical lines represent standard mean errors. Different low case letters indicate statistically significant differences at $p < 0.05$; ns – not significant

Figure 2. Effect of *Nigella sativa* plant residues on height of rigid ryegrass. Vertical lines represent standard mean errors. Different low case letters indicate statistically significant differences at $p < 0.05$; ns – not significant

lower heights (7.49 and 28.61 cm at 19 and 40 DAS, respectively). Moreover, the plant height was also affected by time and the differences between the different residue treatments were observed after 19 DAS.

Concerning the number of tillers, the effect of different plant residues was also found to be statistically significant ($p < 0.001$), especially at 52 DAS where the lowest value (2.3) presented in rigid ryegrass plants treated with the higher rate (20 g) of *N. sativa* shoot residues (Fig. 3a). At 27

DAS, the plant residue treatments had a significant difference with control; however, they did not presented differences among them. The combined analysis of variance revealed that this growth parameter was also affected by time ($p < 0.001$) and an interaction between plant residues and time was observed ($p < 0.05$).

The leaf number was also negatively inhibited ($p < 0.001$) by *N. sativa* plant residues (Tab. 1). From 34 DAS until the end of the experiment (52 DAS), the lowest values (4.1, 5.4 and 6.2 at 34,

Figure 3. Effect of *Nigella sativa* plant residues on a) tiller number and b) leaf number of rigid ryegrass. Vertical lines represent standard mean errors. Different low case letters indicate statistically significant differences at $p < 0.05$; ns – not significant

Figure 4. Effect of *Nigella sativa* plant residues on a) fresh and b) dry weight of above-ground parts of rigid ryegrass. Vertical lines represent standard mean errors. Different low case letters indicate statistically significant differences at $p < 0.05$; ns – not significant

40 and 52 DAS, respectively) presented in pots treated with 20 g of *N. sativa* shoot residues. As in the tillers number measurement, time presented a statistically significant difference ($p < 0.001$) and an interaction between *N. sativa* residues and time was also found ($p < 0.01$).

The above-ground parts fresh weight was actually affected by plant residues of *N. sativa* plant (Tab. 1). In the first measurement, took place at 40 DAS, the lowest fresh weight (0.32 g) was found in plants treated with the double quantity (20 g) of root residues; however, the differences among the high quantity (20 g) residue treatments were not statistically significant (Fig. 4). In the final measurement (52 DAS), the lowest fresh weight

was recorded in 20 g of shoot treatment with the value being 0.66 g. Regarding the dry weight of above-ground parts of rigid ryegrass, the values in all measurements were in relation to those of fresh weight. At the end of the experiment (52 DAS), the lowest value recorded in the case of 20 g of soil-incorporated shoot tissues was 0.15 g.

Conclusions

To sum up, the results of the present study revealed significant weed suppressive effects of *N. sativa* residues incorporated into the soil. The plant residues reduced the seedling emergence and growth parameters of rigid ryegrass as compared to the control. The lowest rate of rigid

Table 1. Combined analysis of variance for *Nigella sativa* plant residue treatment and time effects on seedling emergence and first growth of rigid ryegrass

Source	Seedling emergence		Height		Tiller number	
	df	F	df	F	df	F
Treatment	6	18.08***	6	6.59***	6	5.54***
Time	1	21.64***	5	342.97***	3	77.97***
Treatment X Time	6	0.99 ^{ns}	30	0.99 ^{ns}	18	2.22*

Source	Leaf number		Fresh weight of above-ground parts		Dry weight of above-ground parts	
	df	F	df	F	df	F
Treatment	6	6.38***	6	5.64***	4	3.68***
Time	3	70.76***	1	56.44***	2	97.37***
Treatment X Time	18	2.04*	6	1.47 ^{ns}	8	1.74 ^{ns}

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns – not significant

ryegrass seed germination (38%) was observed in pots treated with 20 g leaf residues. Regarding height, the lowest value (26.7 cm) was found in the case of incorporation of 20 g root residues. The highest quantity (20 g) of shoot residues reduced leaf number, tiller number, fresh and dry aerial biomass up to 58, 51, 64 and 59%, respectively. It can be concluded that the effect of incorporation of *N. sativa* residues depended on the type and amount of residue. *N. sativa* could be utilized as an allelopathic crop to control weeds and future studies should focus on isolating and identifying allelochemicals in roots, shoots and leaves of this species.

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