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Wheat Response and Weed-Suppressive Ability in the Field Application of a Nanoencapsulated Disulfide (DiS-NH_2) Bioherbicide Mimic

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Abstract: Improving the efficacy of bioherbicides to overcome weed resistance phenomena is one of the main challenges within agriculture. Organic encapsulation is attracting attention as an alternative and eco-friendly tool, mainly in organic farming. In this research, for the first time, across three different wheat field trials, we tested the weed-suppressive ability (WSA) and crop response of a nanoparticle formulation of DiS-NH_2 (2,2′-disulfanediyldianiline) applied as post-emergence foliar herbicide, both at standard (T1, 0.75 g m^{-2}) and double dosages (T2, 1.5 g m^{-2}), compared to no weeding (NC) and chemical weed control (PC). Averaged over locations, T2 showed the highest WSA (51.3%), followed by T1 (40.9%) and PC (33.5%). T2 induced also a wheat grain yield and a plant height comparable to PC (3185 kg ha^{-1} and 67.7 cm vs. 3153 kg ha^{-1} and 67.7 cm, respectively). Moreover, compared to NC, T2 increased the number of spikes m^{-2} (+19%) and the number of kernel $spikes^{-1}$ (+26%). Similar results were observed for T1, which caused also a significant reduction in non-vitreous kernels (−40%). These promising results suggest that the nanoencapsulated DiS-NH_2 could be a good candidate as a post-emergence bioherbicide in wheat crop production.

Keywords: allelopathy; nanoformulation; organic encapsulation; organic farming; *Triticum durum*; weed management

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1. Introduction

Wheat, an essential staple food for more than 35% of the world's population, is the most important crop around the world, with a global harvested area of 220 × 10^6 ha producing 770 × 10^6 Mg of grain [1]. Within the European Union (EU), wheat grain production covers 62 × 10^6 ha, yielding 269 × 10^6 Mg [1]. Alongside conventional agriculture, organic farming has gained more and more importance, already covering 14.7 × 10^6 ha in 2020 (+56% than in 2012) in the EU, corresponding to 9.1% of the total utilized agricultural area [2]. Among all member states, Sweden has the largest shares of organic cereals (excluding rice) as a percentage of the total cereal production (7.1%), followed by Estonia (6.1%) and Italy (5.8%) [2].

In wheat organic farming, weeds are recognized by farmers as the greatest yield-limiting constraint, followed by drought, nitrogen supply, and pedo-climatic conditions [3,4]. Indeed, weeds highly compete with wheat plants for water, nutrients, and light, and, in some cases, the competition acts in synergism with the release of allelochemicals into the environment [5]. In conventional agriculture, the inappropriate application of chemicals for herbicidal control over years has caused environmental contamination and has led to the development of herbicide-resistant weeds [6], which are esteemed to increase in the near future [7]. In organic wheat agroecosystems, weed management is more laborious than conventional practices due to the prohibition of herbicide use and the high costs of hand and mechanical weeding. For these reasons, the search for alternative and eco-friendly weed management strategies is an urgent necessity. Among them, following the 'Zero Hunger' goal achievement of the "United Nations Sustainable Development Goals", the application of bioherbicides will become of outstanding importance in the near future [8]. These agents are recognized as compounds from biological sources, their analogues and derivatives, or (living) organisms, which suppress target weed populations without harming the environment [9,10]. Among allelochemicals with potential uses as herbicides, aminophenoxazinones have shown good growth inhibition results against *Bromus japonicus* Thunb., *Chenopodium album* L., *Portulaca oleracea* L., *Avena fatua* L., and *Lolium rigidum* Gaudin [11]. These compounds are degradation products of benzoxazinones, which are usually produced by the Poaceae family, but also by dicot clades such as the Acanthaceae, Ranunculaceae, and Scrophulariaceae families [12]. However, the isolation process and the obtained amount of these natural products are some of the limiting points for high-scale applications. That way, it is possible to synthetize natural product analogs and mimics, which are inspired by those compounds, in a small number of chemical steps to solve the limitation issue. This is the case of DiS-NH_2, which presents a similar phytotoxic profile to that of 2-amino-3*H*-phenoxazin-3-one (APO), a degradation product of benzoxazinones, and similar chemical scaffold changing oxygen atoms by sulfur atoms [13]. This compound has also been tested against weed germination, as well as root and shoot formation, with high inhibition values against *L. rigidum*, *Echinochloa crus-galli* (L.) P. Beauv., and *Urochloa decumbens* (Stapf) R.D. Webster [13,14].

In order to overcome some bioherbicide-related limitations, such as low water solubility, low persistence, and phytotoxic efficiency, the scientific community is nowadays focusing on field-persistent encapsulation structures in relation to allelochemicals and mimics [15]. Unfortunately, the organic encapsulation of allelochemicals has only been tested in in vitro conditions [14,16]. On the contrary, to the best of our knowledge, the biological activities of encapsulated bioherbicides in open fields are still unknown. Therefore, for the first time, we tested the field efficacy of a disulfide bioherbicide mimic (DiS-NH_2) applied as a nanoparticle formulation to investigate the weed-suppressive ability and wheat crop response in terms of morphological, productive, and qualitative traits. We hypothesized that the nanoencapsulated DiS-NH_2 bioherbicide mimic may suppress the weeds across three different wheat fields without damaging the main crop.

2. Materials and Methods

2.1. Formulation of Encapsulated DiS-NH_2

To create DiS-NH_2 (2,2′-disulfanediyldianiline), a similar method to one previously reported was used, with a few adjustments [13]. First, 4 g of 2-aminothiophenol (32 mmol) (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in a mixture of ethanol and water (1:1, 100 mL) and stirred for 24 h while being exposed to air. The resulting yellow precipitate was filtered and washed with water, producing the product with a yield of 80%. After that, this bioactive compound was encapsulated in a core–shell structure generated by Pluronic F-127® (Sigma-Aldrich, St. Louis, MO, USA) (Figure 1). This formulation method generates a polymeric shell around the bioactive compound DiS-NH_2. Polyvinyl alcohol (PVA) was used to stabilize the nanoemulsion, preventing the undesired DiS-NH_2@Pluronic particle agglomeration. The synthetic approach, as previously reported in the literature, was slightly modified [14,17]. The formulation was repeated multiple times with small amounts of DiS-NH_2 (1.0 g). This was dissolved in 96 v% ethanol (50 mL). In a separate vessel, Pluronic F-127® (3.0 g) was dissolved in milli-Q water (150 mL). Additionally, a solution of PVA (20 mg) (Acros Organics, Thermo Fisher Scientific, Waltham, MA, USA) was prepared in water (5 mL).

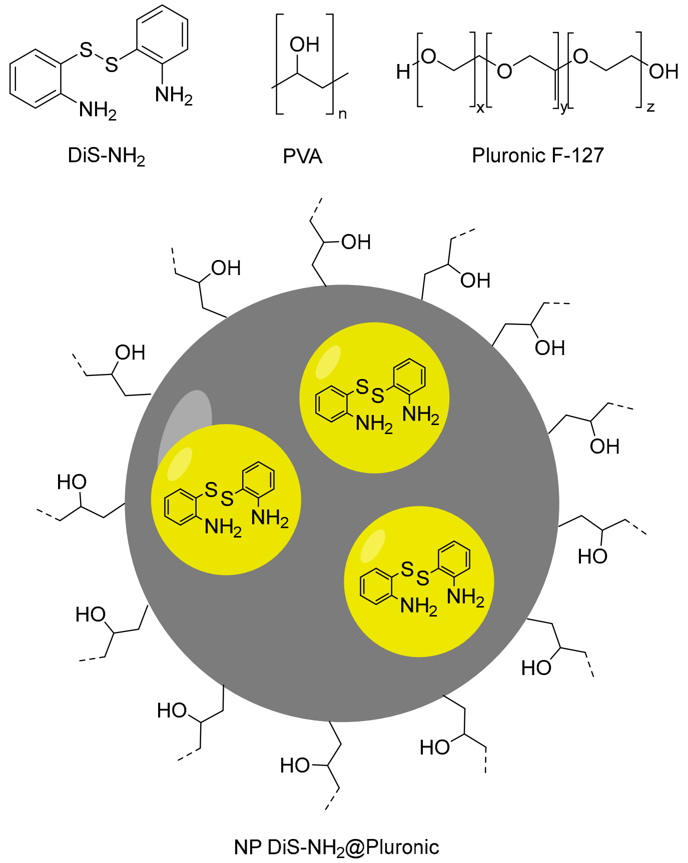


Figure 1. Structure of the bioactive compound (DiS-NH_2), the encapsulating polymer (Pluronic F-127), and the matrix (PVA). NP DiS-NH_2@Pluronic is the proposed nanostructure of the synthesized system.

The stirred solution of Pluronic F-127® was cooled with ice, and the DiS-NH_2 solution was added dropwise through a 0.45 µm nylon membrane. The resulting solution was stirred for 10 min. The PVA solution was then added dropwise and dialyzed for 10 min with type II water using a membrane with a 12,000 Da cut-off (dialysis tubing cellulose membrane, Sigma-Aldrich, Steinheim, Germany) to remove the amount of DiS-NH_2 that was not encapsulated. This solution was frozen with liquid nitrogen to keep the particle size in the nanoscale. This frozen solution, which contained the functionalized nanoparticles, was lyophilized using a LyoAlfa 15 Telstar® machine to produce a fine yellow powder. This method was repeated several times to obtain sufficient material for the field experiments. The percentage of encapsulation was determined by dissolving the NPs (1 mg) in acetonitrile (1 mL). The mixture was agitated in a vortex to break up the particles and release DiS-NH_2. The sample was filtered through a 0.22 µm nylon membrane, and the solution was analyzed using high-performance liquid chromatography (HPLC) to determine the concentration of DiS-NH_2 within the nanoparticles. The UV absorbance was measured with a detector with a wavelength of 260 nm. The HPLC system was VWR Hitachi Chromaster (VWR International, Radnor, PA, USA) with a model 5430 DAD, a model 5310 column oven, a model 5260 autosampler, and a model 5110 solvent pump. A Rever® C18 column (25 × 4.6 mm; Phenomenex, Macclesfield, UK) with a 5 µm pore size was utilized. Solvents were obtained from VWR and filtered using a Durapore® 0.4 µm filter membrane (3 M, Saint Paul, MN, USA) before use in the HPLC apparatus. The retention time of DiS-NH_2 was 4.67 min in isocratic mode (30% MeOH: H_2O). The areas of the peaks were converted to concentrations using a calibration curve obtained from previously reported methods [14].

This formulation was designed according to previous specifications and reported results shown by Mejías et al. [17]. Pluronic F-127® nanoparticles functionalized with PVA have already shown good results against parasitic plant germination such as *Phelipanche aegyptiaca* (Pers.) Pomel and *P. ramosa*. Furthermore, in vitro studies on the innocuous effect of empty nanoparticles and synthetic components have also been demonstrated with *P. oleracea*, *Plantago lanceolata* L., and *L. rigidum* [14].

The JEOL2100 transmission electron microscope was used for electron microscopy analysis. To prepare the samples, a drop of the sample dispersed in type-I water was added to a lacey-carbon-coated 300 mesh copper grid. The prepared TEM grid was left to dry on filter paper overnight to remove the water before being loaded into the electron microscope. The recording mode used was 200 KeV. Energy-dispersive spectroscopy (EDXS) was performed using an EDAX Octane Ultra SDD detector, which has a sensitive area of 100 mm^2 and a collection solid angle of 1.1 sr. The images were captured using both high-angle annular dark-field (HAADF) and bright-field (BF) modes, as shown in Figure 2.

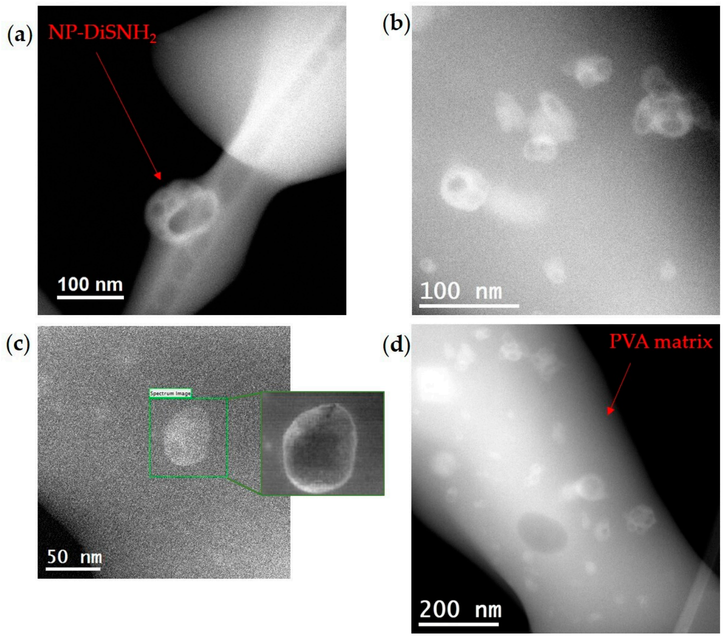


Figure 2. (**a**) STEM image at the HAADF mode to observe the nanoparticle multi-cavity structure generated by Pluronic F-127®. (**b**) STEM image at the HAADF mode with several NPs with similar sizes and shapes. (**c**) HAADF zoom to observe the shape of the NPs. (**d**) STEM image at the HAADF mode with medium magnification to observe the PVA matrix where the NPs are embedded.

2.2. Field Trials and Crop Management

This experiment was carried out from December 2021 to June 2022 and replicated across three locations (locations I, II, and III) sited in central Sicily (south Italy), in a narrow area of about 40 km which lies between Butera (Caltanissetta, location I) and Piazza Armerina (Enna, locations II and III). This area covers Regosoils, Typic Xerorthensis, or Xerochrepts [18], characterized by poor organic matter content, clayey texture, alkaline reactions, and high exchangeable K_2O levels. Climate is semi-arid Mediterranean, with an average annual rainfall of 500 mm (concentrated in the autumn period) and hot–dry summers.

The experiment comprised 4 treatments arranged into randomized blocks, with 3 replications for each treatment. Overall, 12 plots (4 treatments × 3 replicates) for each location were combined, giving a total net experimental area of 144 m^2 (4 m^2 plot size × 36 plots). Treatments consisted of nanoencapsulated DiS-NH_2 bioherbicide mimic applied at standard (T1) and double (T2) dosages, a control without any weeding practice (NC) and a chemical weed control (PC). A border 2 m width was considered to separate each plot.

In location I, chemical control was performed by spraying Atlantis® WG (mesosulfuron-methyl + iodosulfuron-methyl-sodium + mefenpyr-diethyl) at 0.05 g m^{-2} and Buctril® Universal (bromoxynil + 2,4-D) at 1×10^{-4} L m^{-2} during wheat tillering (growth stage 23 of the BBCH scale) [19]. Traxos® Pronto 60 (pinoxaden + clodinafop-propargyl + cloquintocet-mexyl) and Manta® Gold (fluroxypyr + clopyralid + MCPA), both at 2×10^{-4} L m^{-2}, were mixed with Amadeus® Top (thifensulfuron-methyl + tribenuron-methyl) at 3×10^{-3} g m^{-2} for chemical control in locations II and III. Herbicide dosages were those recommended by the producers. The nanoencapsulated DiS-NH_2 bioherbicide mimic was used as post-emergence foliar spray by means of a knapsack hand sprayer, during wheat tillering (on 28th February in location I and 7th March in locations II and III), at the following the amounts: 0.75 g m^{-2} (T1) and a double dosage of 1.5 g m^{-2} (T2). It has to be consider that only 21% of the nanoencapsulated DiS-NH_2 belongs to the active ingredient, and the rest are the formulation excipients. Therefore, 0.1575 g m^{-2} and 0.315 g m^{-2} of the bioactive ingredients were used at T1 and T2, respectively. Both the herbicides and the nanoencapsulated DiS-NH_2 bioherbicide mimic were applied with 500 L water ha^{-1}.

Durum wheat [*Triticum turgidum* subsp. *durum* (Desf.) Husn.] cv. 'Core' (Platani/Gianni) was sown by hand on the second half of December 2022 at 200 kg seeds ha^{-1} across the three locations. Core is a modern wheat cultivar, characterized by medium and early maturity and good resistance to both low winter temperatures and *Fusarium* spp. The three experimental fields were cultivated following the local agronomic management [20]. In detail, 20 cm ploughing was carried out in early autumn followed by disk harrowing to prepare the seedbed. Before sowing, 120 Kg ha^{-1} of diammonium phosphate (18% N–46% P_2O_5) was soil-incorporated. Furthermore, 60 kg N ha^{-1} of ammonium nitrate (27%) was then applied in the post-emergence stage. To control fungal diseases, Mirador® SC (azoxystrobin) was sprayed at 1 L ha^{-1} in all plots and all locations during the flowering stage.

2.3. Measurements

2.3.1. Weed-Suppressive Ability (WSA) of Weedy Treatments

The WSA was calculated into three 1 m^2 fixed quadrats for each treatment, chosen without considering the borders of each plot, according to the following formula:

$$\mathrm{WSA} = \left(\frac{W_c - W_t}{W_c}\right) \times 100 \tag{1}$$

where W_c is the aboveground dry weed biomass of NC and W_t is the aboveground dry weed biomass of T1, T2, or PC. Dry weights were determined after oven-drying at 55 °C up to a constant weight until all weeds were present in each quadrat [21]. The WSA was assessed on 11th April and 15th June for each treatment and location in order to intercept a complete spectrum of the weed flora. The WSA is a percentage that indicates the ability to suppress weed growth and reduce weed seed production [22], with positive and negative values indicating inhibition and stimulation, respectively.

2.3.2. Wheat Morphological, Productive, and Quality Traits

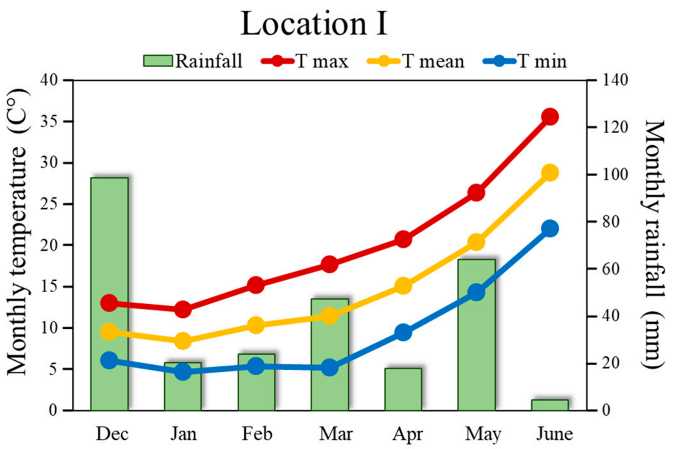
The following durum wheat parameters were considered on four representative quadrats of 1 m^2 in each treatment: plant height, grain production, spikes m^{-2}, kernel $spike^{-1}$, thousand kernel weight, harvest index, non-vitreous kernels, and skimpy kernels. The plant height and number of spikes were measured at the earing stage: the former on ten plants per quadrat from the soil surface to the top of the spike and the latter by counting all the spikes contained in each quadrat. After grain ripening, the plants were hand-harvested on 12th June in location I and on 15th June in locations II and III. The number of kernels per spike was determined by counting the kernels on every spike from a subsample of 10 plants. The grain yield was obtained by referring to a unit area (4 m^2) and correcting to a 13% moisture basis. The harvest index, i.e., the relationship between the dry weight of kernels and the dry weight of the total biomass produced by the plant, was calculated as [23]:

$$\text{Harvestindex} = \frac{\text{kerneldryweight}}{\text{straw} + \text{spikedryweights}} \tag{2}$$

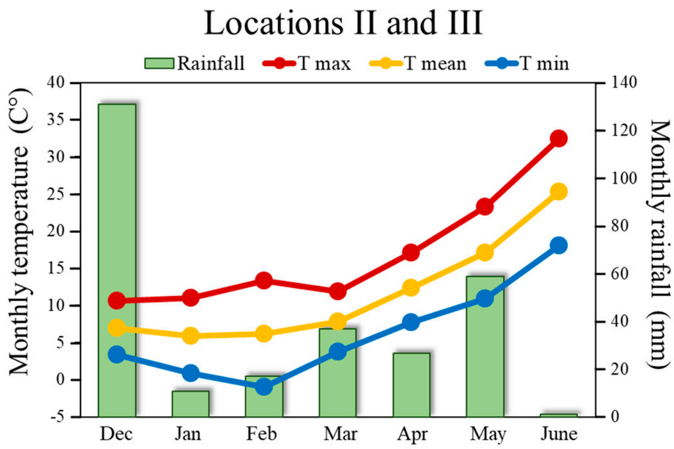
The thousand kernel weight and the incidence (%) of non-vitreous kernels and skimpy kernels were determined on three sets of 100 kernels per plot for the former and on three 15 g sets of kernels for the latter quality traits.

2.4. Climatic Data

A meteorological station (Mod. Multirecorder 2.40; ETG, Firenze, Italy) was used to daily monitor rainfall and air temperature across the three locations. The total rainfall was 276 mm in location I and 282 mm in locations II and III (Figure 3). Of these amounts, about 45% fell between March and May, contrariwise to the normal trend of the zone. December recorded the highest rainfall level at all locations. Air temperatures allowed a suitable growing cycle for durum wheat, except February that registered a minimum temperature below 0 °C at locations II and III. The mean air temperature was ~2 °C higher in location I over the whole experimental period.



(**a**)



(**b**)

Figure 3. Climatic trends during the experimental period (December 2021–June 2022) at location I (**a**) and locations II and III (**b**), sited in Central Sicily (Italy).

2.5. Statistical Analysis

To analyze the effect of the nanoencapsulated DiS-NH_2 bioherbicide mimic on durum wheat, a two-way analysis of variance (ANOVA) was conducted using the CoStat® 6.003 software (CoHort, Monterey, CA, USA). In this linear model, treatments and locations were treated as fixed effects. The WSA was initially analyzed with a three-way general linear model (4 treatments × 3 locations × 2 sampling times), but since ANOVA revealed a non-significance for the sampling time, this factor was then pooled over treatments and locations. Differences between the means were compared using Fisher's protected least significant difference (LSD) test at the 5% significance level. To obtain a homogeneous distribution of the variances, percentage values (WSA, n-VK, and SK) were arcsine$\sqrt{\%}$ transformed. Bartlett's test was run to assess the homoscedasticity. The Pearson product–moment correlation coefficient (r) was also calculated to evaluate the correlation between the aboveground weed biomass and the durum wheat yield.

3. Results

3.1. Weed-Suppressive Ability of Encapsulated DiS-NH_2

The ANOVA applied on the weed-suppressive ability data revealed a significance value of $p \leq 0.001$ for the location and a significance value of $p \leq 0.05$ for treatment and their interaction (Table 1). Overall, compared to no weeding (NC), high and positive WSA indices were found for all treatments and locations. In location I, the nanoencapsulated DiS-NH_2 bioherbicide mimic applied at both standard (T1) and double (T2) dosages showed the same WSA of the chemical control (PC), whereas in location II and III, T2 had a +71% and +70% WSA compared to PC, respectively (Figure 4). T1 did not differ statistically from PC in any of the three locations, although a +56% WSA was observed at location II. We analyzed the average WSA values of all locations, which showed the highest WSA (51.3%) for T2, followed by T1 (40.9%) and PC (33.5%) (Figure 4). These results were corroborated by the data on aboveground weed biomass, which, based on the average of locations, showed a 160.5 g dry weight (DW) m^{-2} for NC, a 83.8 g DW m^{-2} for T1, a 69.2 g DW m^{-2} for T2, and a 96.3 g DW m^{-2} for PC. We then compared the locations, which indicated the highest WSA at location I (56.4%), followed by location II (37.7%) and III (31.6%).

Table 1. *F*-values of the main effects and their interactions resulting from two-way analysis of variance (ANOVA) on the morphological and productive traits of durum wheat.

Trait	Source of Variation				CV
	Blocks	**Location (L)**	**Treatment (T)**	**(L) × (T)**	
Degrees of freedom	2	2	3	6	
Weed-suppressive ability	0.3 ns	9.4 ***	4.5 *	2.7 *	42.8%
Plant height	0.1 ns	219.6 ***	7.2 **	2.4 ns	3.6%
Grain yield	0.9 ns	75.9 ***	13.6 ***	9.3 ***	12.6%
Spikes m^{-2}	0.9 ns	7.6 **	4.8 *	2.0 ns	14.4%
Kernel spikes^{-1}	0.3 ns	7.6 **	10.3 ***	1.4 ns	10.0%
Thousand kernel weight	2.0 ns	13.0 ***	2.1 ns	1.5 ns	7.5%
Harvest index	1.1 ns	50.4 ***	3.1 *	2.9 *	9.2%
Non-vitreous kernels	0.6 ns	34.2 ***	10.8 ***	2.0 ns	10.7%
Skimpy kernels	1.6 ns	1.2 ns	5.1 **	5.7 ***	11.7%

Values are given as *F* of Fisher; ***, **, and * indicate significance at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively. ns: not significant. CV: coefficient of variation.

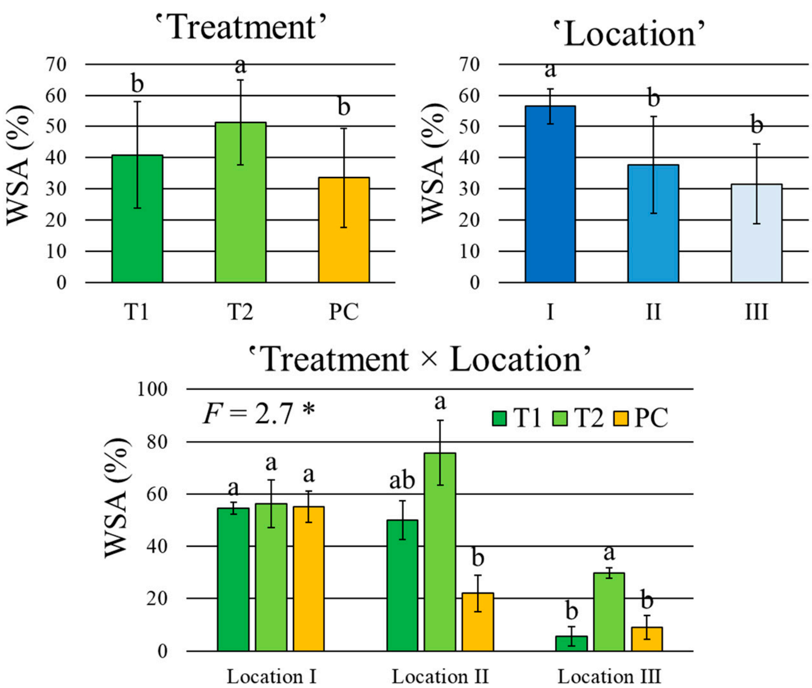


Figure 4. Weed-suppressive ability (WSA) of DiS-NH_2 bioherbicide mimic on durum wheat crop in relation to main effects (treatment and location) and their interactions. Bars represent the standard deviation (n = 3). Different letters and * indicate the statistical significance at $p \leq 0.05$ (Fisher's LSD test). T1: DiS-NH_2 at a standard dosage (0.75 g m^{-2}); T2: DiS-NH_2 at a double dosage (1.5 g m^{-2}); NC: negative control (no weeding); PC: positive control (chemical weeding).

3.2. Wheat Height

The treatments showed a $p \leq 0.01$ significance level for the plant height, although location was the main contributor to the overall variance (95.8%), while their interaction was not significant (Table 1). Compared to NC, T1 and T2 statistically increased the plant height at the same extent of PC (Table 2). As suggested by the ANOVA, this characteristic was highly affected by location, and the following trend was observed: location III > location II > location I.

Table 2. The effect of treatment and location on durum wheat plant height (Ph), grain yield (GY), number of spikes (nS), number of kernels (KS), thousand kernel weight (Tkw), harvest index (HI), non-vitreous kernels (n-VK), and skimpy kernels (SK).

	Ph	GY	nS	KS	Tkw	HI	n-VK	SK
	cm	kg ha^{-1}	no. m^{-2}	no. spike^{-1}	g		%	
Treatment								
T1	66.1 (3.7) ^{a}	2732.2 (361.7) ^{b}	230.7 (50.4) ^{bc}	40.0 (4.1) ^{a}	48.3 (5.4) ^{a}	0.45 (0.1) ^{a}	12.4 (5.3) ^{c}	16.5 (2.6) ^{a}
T2	67.7 (3.8) ^{a}	3185.0 (357.4) ^{a}	249.6 (38.6) ^{ab}	40.6 (4.3) ^{a}	45.2 (3.9) ^{ab}	0.46 (0.1) ^{a}	19.1 (2.9) ^{ab}	15.2 (1.5) ^{a}
NC	63.1 (3.8) ^{b}	2236.0 (239.4) ^{c}	209.5 (45.5) ^{c}	32.1 (4.7) ^{c}	46.0 (5.6) ^{ab}	0.43 (0.1) ^{ab}	20.8 (4.1) ^{a}	16.8 (4.4) ^{a}
PC	67.7 (4.6) ^{a}	3153.3 (290.2) ^{a}	268.3 (39.6) ^{a}	35.7 (4.8) ^{b}	44.3 (4.4) ^{b}	0.41 (0.1) ^{b}	17.1 (5.6) ^{b}	11.4 (3.5) ^{b}
Location								
I	55.4 (2.3) ^{c}	1853.6 (219.8) ^{c}	210.3 (38.6) ^{b}	38.9 (5.4) ^{a}	41.7 (4.4) ^{b}	0.35 (0.0) ^{c}	11.2 (3.9) ^{b}	16.2 (5.7) ^{a}
II	67.3 (3.6) ^{b}	2981.2 (306.1) ^{b}	264.9 (49.2) ^{a}	38.7 (3.8) ^{a}	48.6 (4.4) ^{a}	0.51 (0.0) ^{a}	20.5 (4.1) ^{a}	15.0 (4.6) ^{a}
III	75.9 (3.5) ^{a}	3645.1 (410.7) ^{a}	243.4 (39.7) ^{a}	33.7 (5.8) ^{b}	47.5 (2.9) ^{a}	0.45 (0.1) ^{b}	20.4 (5.3) ^{a}	13.8 (3.6) ^{a}

Values are means (n = 4) with standard deviation (in brackets). Values within a column followed by different letters are significantly different at $p \leq 0.05$ (Fisher's LSD test). T1: DiS-NH_2 at a standard dose; T2: DiS-NH_2 at a double dose; NC: negative control (no weeding); PC: positive control (chemical weeding).

3.3. Wheat Yield and Productive Traits

The two-way ANOVA highlighted a $p \leq 0.001$ effect of the 'location $\times$ treatment' interaction for grain yield, and, comparable to plant height analysis, the majority of the variance was explained by location (76.1%) (Table 1). The application of the nanoencapsulated DiS-NH_2 bioherbicide mimic at a double dosage (T2) led to significant improvements in the wheat grain yield with respect to NC in all locations (+57.6% in location I, +17.6% in location II, and +19.8% in location III) (Figure 5). In locations I and II, furthermore, T2 induced a higher grain yield than PC (+37.0% and +18.9%, respectively). Except for location III, T1 plots also showed a similar grain yield to PC (1824.4 vs. 1714.1 kg ha^{-1} in location I and 2970.1 vs. 2756.8 kg ha^{-1} in location II). Averaged over the various locations (Table 2), T2 resulted in a grain yield comparable to PC (3185.0 and 3153.3 kg ha^{-1}), followed by T1 (2732.2 kg ha^{-1}), and, to a lesser extent, NC (2236.0 kg ha^{-1}). As observed for plant height data, the trend location III > location II > location I was observed as well.

With regards to the yield components, except for the number of kernels per spike, all characters were mostly affected by location (Table 1). In fact, it covered 49.7%, 69.9%, and 87.5% of the variability for the number of spikes, the thousand kernel weight, and the harvest index, respectively. The two-way interaction was significant for the harvest index ($p \leq 0.05$). The main effects are displayed in Table 2. With some exceptions, the application of the nanoencapsulated DiS-NH_2 bioherbicide mimic increased the wheat productive traits. In particular, with respect to NC, T1 caused a +10.1% increase in spikes m^{-2} and a +24.5% increase in the kernel spikes^{-1}. Similarly, T2 enhanced the spikes m^{-2} by 19.1% and the kernel spikes^{-1} by 26.5%. The chemical control (PC) determined the highest number of spikes (268.3 m^{-2}). Excluding the kernel spikes^{-1}, location I experienced the lowest value for all productive traits.

Thousand kernel weight was the only yield component that was not affected by treatment (Table 1). If compared to NC, T1 and T2 showed non-significant differences, while PC decreased the thousand kernel weight by 3.7% (Table 2). Moreover, the harvest index was slightly influenced by treatment. No significant differences between treatments emerged neither in location I nor in location II, while PC caused a significant reduction (−21.9% than

NC) in the harvest index in location III (Figure 6). For both the thousand kernel weight and the harvest index, the location III > location II > location I trend was observed.

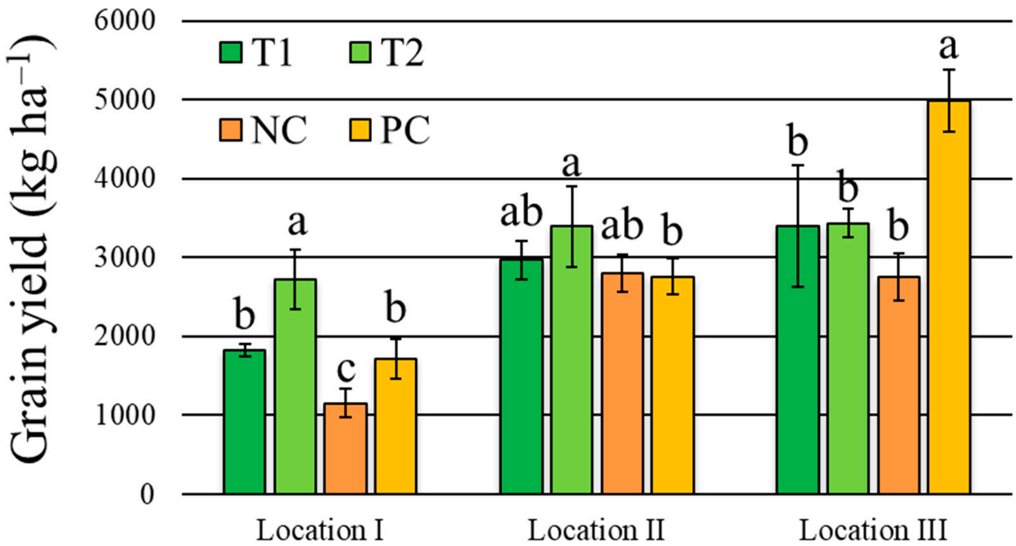


Figure 5. 'Location × treatment' interaction derived from two-way analysis of variance (ANOVA) on durum wheat grain yield. Bars represent the standard deviations (*n* = 4). Different letters indicate statistical significance at $p \leq 0.05$ (Fisher's LSD test). T1: DiS-NH_2 at a standard dosage (0.75 g m^{-2}); T2: DiS-NH_2 at a double dosage (1.5 g m^{-2}); NC: negative control (no weeding); PC: positive control (chemical weeding).

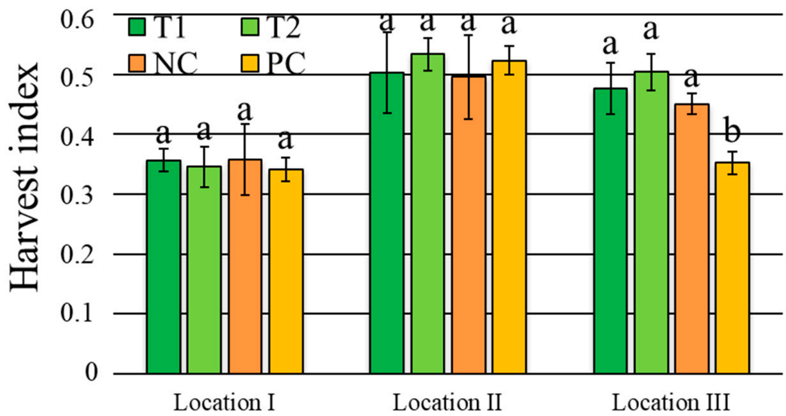


Figure 6. Location × treatment interaction derived from two-way analysis of variance (ANOVA) on the durum wheat harvest index. Bars represent the standard deviations (*n* = 4). Different letters indicate statistical significance at $p \leq 0.05$ (Fisher's LSD test). T1: DiS-NH_2 at a standard dosage (0.75 g m^{-2}); T2: DiS-NH_2 at a double dosage (1.5 g m^{-2}); NC: negative control (no weeding); PC: positive control (chemical weeding).

3.4. Wheat Grain Quality Traits

Non-vitreous kernels and skimpy kernels had an opposite trend based on the ANOVA: the former was mostly affected by location (71.7%), whereas the latter was mostly affected by treatment (37.5%) (Table 1). In both cases, NC caused the highest incidence of non-vitreous kernels (20.8%) and skimpy kernels (16.8%) (Table 2). Compared to NC, T1 significantly reduced the incidence of non-vitreous kernels (−40.4%), while a significant reduction in skimpy kernels was observed in PC plots only. For this quality trait, the two-way interaction was highly significant and pointed out the instable behavior of treatments across locations (Figure 7). In location I, the incidence of skimpy kernels was markedly higher in NC plots than the other treatments; in locations II, T1 and T2 significantly increased the incidence compared to both NC and PC; in location III, no significant differences were observed between T1, T2, and NC.

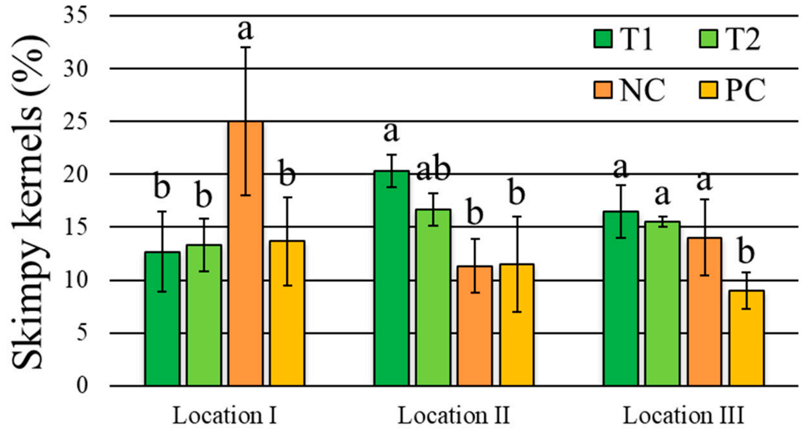


Figure 7. Location × treatment interaction derived from two-way analysis of variance (ANOVA) on durum wheat grain skimpy kernels. Bars represent the standard deviations (*n* = 4). Different letters indicate statistical significance at $p \leq 0.05$ (Fisher's LSD test). T1: DiS-NH_2 at a standard dosage (0.75 g m^{-2}); T2: DiS-NH_2 at a double dosage (0.75 g m^{-2}); NC: negative control (no weeding); PC: positive control (chemical weeding).

4. Discussion

The nanoencapsulated DiS-NH_2 bioherbicide mimic showed a considerable WSA on wheat crop weeds, especially when applied at a 1.5 g m^{-2} dosage. This was demonstrated across three different locations, with an average WSA of 51.3%. Interestingly, the 0.75 g m^{-2} dosage also suppressed weeds on a par with weed chemical control. The weed communities under study were dominated by Asteraceae members, spring–summer and annual therophytes, as expected in semi-arid agroecosystems [24]. In particular, major weeds were *Centaurea napifolia* L., *A. fatua*, *Glebionis coronaria* s.l., *Fumaria officinalis* L., *Sinapis arvensis* L., and *L. multiflorum* in location I; *G. coronaria*, *S. arvensis*, *Papaver rhoeas* L., *Dactylis glomerata* L., and *Anethum graveolens* L. in location II; *L. perenne*, and *Medicago polymorpha* L., *A. fatua*, and *S. arvensis* in location III. Therefore, both broadleaf and grass weeds were controlled by the DiS-NH_2 bioherbicide mimic, denoting a broad-spectrum weed control. These results corroborated the in vitro findings of Mejías et al. [15], where the polymeric nanoparticles of disulfide were found to strongly inhibit *Plantago lanceolata* L. seed germination and wheat coleoptile elongation. In another study, seven aminophenoxazinone derivatives showed an important root length reduction in the *P. oleracea* weed, with a phytotoxicity similar to, or even higher than, the herbicide pendimethalin [25]. This may be a result of the postulated mode of action, since histone deacetylases (HDAs) and histone acetyltransferases are assumed to be the main targets of the DiS-NH_2 bioherbicide and analogues, i.e., two important enzymes involved in the change from the vegetative to the reproductive form, thus causing a reduced plant development [26]. Specifically, this kind of compound may interact with the zinc cofactor which regulates the process in some HDAs, such as HDA2 and HDA6. Indeed, no symptoms on weeds were observed in this study; we only detected a significant reduction in plant development. Furthermore, the nanoparticle formulation proposed here allowed a stable WSA over the three locations, and, to the best of our knowledge, this is the first report of a multi-trial field application of an organic encapsulated agent. The suppression of weeds is not only important for the current growing season, but it also provides long-term benefits due to the reduction in weed seed production and consequently the progressive decrease in the weed soil seedbank. On the contrary, the low efficacy of PC in locations II and III may be attributable to a two-fold motivation: the long-term application of the used active ingredients that probably increased weed resistance and the presence of major weeds not detected in location I (namely *P. rhoeas*, *D. glomerata*, *A. graveolens*, and *M.polymorpha*).

In addition to the WSA, the DiS-NH_2 bioherbicide mimic did not exert any phytotoxic effects on the wheat crop after its application (by visual evaluation), as also demonstrated by the higher plant heights, yields, and yield components shown for wheat plants subjected to T1 and T2 compared to NC plots. The plant height, grain yield, spike number m^{-2}, thousand kernel weight, and harvest index values were in the range for similar durum wheat varieties [23,27,28]. The improved morphological, productive, and qualitative traits were probably caused by the reduction in weed pressure towards wheat, or by its higher weed tolerance (WT). Weed competitiveness, i.e., the ability of a crop to suppress and tolerate weeds, relies on two factors: the WSA and the WT, which is the yielding ability despite weed competition [29]. WSA and WT are often positively correlated, but high WSAs do not always guarantee high yields [30]. Here, we suppose that the DiS-NH_2 bioherbicide mimic decreased the weed competitiveness' and consequently positively influenced the yield wheat response. Indeed, a negative and significant relationship was found between the aboveground weed biomass and the durum wheat grain yield ($r = -0.848$, $p = 0.032$), indicating that the higher the weed biomass is, the lower the wheat grain yield is. Durum wheat grain yield, similar to other grain crops, derives from an optimum balance of three main yield components: the number of spikes per unit area, the number of kernels per spike, and the average weight of kernels [31]. According to García del Moral [32], under Mediterranean conditions, these yield components have an interdependent effect and are able to compensate one another in order to stabilize the yield. Thousand kernel weight was not affected by treatments. The low levels found in T2 and PC may be attributable to their high kernel spike^{-1} number, which indicates greater intra-kernel competitiveness.

5. Conclusions

Our study partially fills a gap in the research topic of plant-based bioherbicides, as we tested the organic encapsulation of plant allelochemical mimics under field conditions for the first time. From this research, it became clear that the nanoencapsulated DiS-NH_2 bioherbicide mimic not only highly suppressed weeds, but also exerted positive effects on wheat crop production. The nanoparticle formulation proposed in the study provided stable results across three different locations, demonstrating its efficiency as valid post-emergence and contact bioherbicide for wheat crops. These results are of greater importance if considering their application in organic farming. In future steps, we aim to test the nanoencapsulated DiS-NH_2 bioherbicide mimic on other crops and under different climatic conditions. Application times and dosages require also further attention.

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