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Article

Trichoderma spp. and Mulching Films Differentially Boost Qualitative and Quantitative Aspects of Greenhouse Lettuce under Diverse N Conditions

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Abstract: The global increasing demand of lettuce is pushing farmers to boost their production through several technical means, including mulching and nitrogen fertilization. However, from an environmental protection perspective, the role of scientific research is to limit the excessive use of some chemical approaches. This research aims to evaluate the possible effects of two mulching films (black polyethylene, PE, and brown photoselective film, BF) and two treatments with a plant growth-promoting product, containing *Trichoderma* spp., (non-treated, - Control and treated with RYZO PEP UP, - TR), on the productive and qualitative traits of lettuce grown under four regimes of nitrogen (0, 30, 60 and 90 kg ha^{-1}, N0, N30, N60, and N90, respectively). The marketable yield increased at higher nitrogen levels, but without differences between the N60 and N90 doses. The photoselective film elicited marketable yield, with an 8% increase over PE. N fertilization also improved photochemical efficiency (higher Soil Plant Analysis Development and chlorophyllous pigments biosynthesis), as well as antioxidant activities (lipophilic—LAA and hydrophilic—HAA) and bioactive compounds (phenols and total ascorbic acid—TAA). Interestingly, *Trichoderma* spp. had a positive effect on these qualitative parameters, especially when combined with mulching films, where the increase generated by PE-TR treatment over the all other treatments was 16.3% and 16.8% for LAA and HHA, respectively. In all treatments, the nitrate leaves content was consistently always within the legal limit imposed by the European community. Overall, although *Trichoderma* spp. did not engender a marked effect on yield, probably due to the short crop cycle, its positive effect on some quality traits is an interesting starting point for further research.

Keywords: mulching film; *Trichoderma*; *Lactuca sativa* L.; nitrogen dose; nutritional quality; yield; sustainability

1. Introduction

Lettuce is the first fresh vegetable cultivated and commercialized in the world, especially in temperate and subtropical regions [1]. The increasing consumption of lettuce is driving farmers to intensify the use of chemical means in agricultural production, therefore not consistently achieving sustainable agriculture principles. In recent years, one of the used methods for boosting food production is plastic mulching films, which allow reaching higher yield in respect to bare soil, both under greenhouse or open-air conditions [2]. In fact, applying mulching films has pointed out several positive factors such as increase of soil temperature, moisture conservation, weeds and pests reduction, higher crop yields, and more efficient use of soil nutrients [3,4]. In agriculture, black polyethylene plastic films are normally used, but the photoselective plastic films have gained important consideration. In several studies, these films have showed very convincing results and performance in terms of quality and quantity of crop yields [5–7]. The traditional black PE film assure the mulching effect, blocking the solar radiation including the Photosynthetically Active Radiation (PAR). Instead, the photoselective films block only the PAR, hence exploiting the useful part of solar radiation to heat or "cool" the soil, based on the optical properties of each film [8].

In order to reach higher production, many crops including leafy vegetables require large quantities of nitrogen, even though its high nitrogen availability does not always correlate with a higher quality of the product [9]. Indeed, an overuse of this macronutrient can induce nitrate accumulation in leaves [10–12] with possible deleterious effects on human health [13]. Moreover, an excessive use of nitrogen can have a detrimental impact on the environment due to nitrate leaching into the groundwater and to greenhouse gas emissions [14,15]. Therefore, in order to overcome these adversities, it is necessary to adopt an adequate management of nitrogen fertilization through an equitable application of the requested dose, as well as to choose the convenient chemical form and application time [16].

Recently, many studies have proposed an eco-friendly approach to improve crop yield, which is the integrated use of plant biostimulants (PBs), especially the use of fungi, such as *Trichoderma*. These plant growth-promoting microorganisms improve pathogen/pest control, increase nutrient uptake, and stimulate photosynthesis and carbohydrate metabolism processes, consequently influencing positively crop productivity and quality [17,18].

Some authors [19–23] found that *Trichoderma* spp. acts as plant biostimulants, improving nutrient uptake, plant growth, and plant tolerance to abiotic stress. Moreover, Colla et al. [23], Fiorentino et al. [22], and Rouphael et al. [21] also demonstrated that the use of *Trichoderma* enhances nutrient-use efficiency (NUE) in lettuce, favoring the N uptake especially under sub-optimal nitrogen conditions.

Based on the abovementioned, several studies have been conducted regarding these factors, but their interaction is still not investigated. Thus, the aim of this research was to evaluate the possible effects of different mulching films and the application of *Trichoderma* as plant growth-promoting fungi on productive and qualitative traits of lettuce grown under different nitrogen regimes.

2. Materials and Methods

2.1. Experimental Setting, Design, and Plant Material

The trial was carried out during winter 2019–2020 in the soil of a polyethylene greenhouse at the experimental site "Gussone Park" of Department of Agricultural Science-DIA in Portici, Southern Italy. The tested crop was lettuce (*Lactuca sativa* L. var. *capitata*) cv. Jumper (Gautier Seed, Cesena, Italy), a plant with a semi-closed head and medium green brilliant leaves.

The design was a factorial comparison between (1) two mulching films, a brown photoselective film (BF) and a black polyethylene (PE); (2) two plant growth promoting treatments (non-treated, —Control and treated with RYZO PEP UP, - TR); (3) four increasing levels of nitrogen fertilization (0, 30, 60, and 90 kg ha^{-1}, N0, N30, N60, and N90, respectively). The optimal nitrogen dose was calculated by the balance method and it resulted 60 kg ha^{-1}. This method takes into account (i) the inputs, such as chemical soil fertility, available nitrogen from the mineralization of organic matter, rain, nitrates in irrigation water, and nitrogen released by previous crops, and (ii) the outputs, mainly crop uptakes, but also the leaching and the volatilization; therefore their difference gives the value of nitrogen needed.

The experimental design was a split plot design with three replications; experimental units were 48 and each one was 6 m long and 0.4 m large. The experimental soil was sandy loam, with neutral pH, a total nitrogen content of 1.2 g kg^{-1}, high content of potassium (1800 mg kg^{-1}) and phosphorus (85 mg kg^{-1}), and a good content of organic matter (1.7%).

2.2. Plant Management, Nitrogen Fertilization, and Trichoderma Application

In mid-January 2020, the mulching films were placed manually; then, on 21 January, the plants were immersed in a water solution with RYZO PEP UP at 1 mL L^{-1}, and the following day, the plants were transplanted at a density of 16 plants per square meter. RYZO PEP UP is a microbial biostimulant produced by Samagri SRL (Cava de' Tirreni, Salerno, Italy) containing *Trichoderma* spp. with a final concentration of 1×10^8 CFU. Moreover, lettuce plants were sprayed directly on the soil surroundings with a solution containing 3 mL L^{-1} of RYZO PEP UP, two times on a two-week basis starting 15 days after the sowing.

According to the experimental design, nitrogen was added as ammonium nitrate (26%) in a single operation two weeks after the transplant at the soil surface, respecting the dose calculated by the balance method. The water losses were calculated by the Hargreaves formula and were fully restored by irrigation.

2.3. Plant Growth Parameters, Marketable Yield, SPAD index, Leaf Colorimetry, and Nitrate Determination

Harvesting was done on three different dates (10, 12, and 17 March), when lettuce heads reached the commercial size. Ten plants per experimental plot (replicate) were harvested, the head diameter was measured, and the leaves were counted; all data were averaged and expressed as the mean of the three replicates. The marketable yield was expressed in tons per hectare. Before harvesting, the measurements of Soil Plant Analysis Development (SPAD) index were made by a portable chlorophyll meter SPAD-502 (Konica Minolta, Tokyo, Japan). On five fully expanded young leaves of five heads per each replicate, the color space parameters were measured by a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan) according to the Commission international de l'eclairage (CIELAB).

A representative fresh sample per replicate was stored at −80 °C for qualitative analysis. Meanwhile, another sample of each replicate was dried in oven at 70 °C until reaching constant weight, in order to be used afterwards for measuring the sum of nitrate and nitrite of NH_4Cl-buffer extract, by Foss FIAstar 5000 continuous flow analyzer. The method is based on the reduction of nitrate to nitrite on a cadmium reductor. The concentration of nitrite is negligible in most cases in comparison with nitrate concentration.

2.4. Chlorophyllous Pigments, Carotenoids and Bioactive Molecules Analysis

Chlorophyllous pigments and carotenoids were measured on 1 g of fresh sample based on the method of Lichtenhaler and Wellburn [24]. The samples were extracted with ammionacal acetone; then, through a spectrophotometer (Hach DR 2000, Hach Co., Loveland, CO, USA), the solution absorbances of chlorophyll a, chlorophyll b, and carotenoids were measured at 662, 647, and 470 nm, respectively, where total cholorphyll is the sum of chlorophyll a and b. The values were expressed as mg g^{-1} fresh weight (fw). The method of Kampfenkel et al. [25] was used for measuring total ascorbic acid content (TAA), which was expressed as mg ascorbic acid 100 g^{-1} fw. Meanwhile, the total phenolic content was determined by the Singleton et al. procedure [26] and expressed as mg gallic acid per 100 g^{-1} dry weight (dw).

2.5. Antioxidant Capacity Analysis

On 200 mg extract of freeze-dried leaves prepared through a freeze drier (Christ, Alpha 1-4, Osterode, Germany), the hydrophilic (HAA) and lipophilic (LAA) antioxidant activity were assessed by the N, N-dimethyl-p-phenylenediamine (DMPD) method [27] and ABTS (2,20-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) method [28], respectively. The values were expressed as mmol ascorbic acid 100 g^{-1} dw for HAA and mmol of Trolox 100 g^{-1} dw for LAA.

2.6. Statistical Analysis

All data were analyzed by the SPSS software package (SPSS version 22, Chicago, IL, USA), using a general linear model (three-way ANOVA). Means were separated according to the Duncan Multiple Range Test (DMRT; significance level 0.05).

3. Results and Discussion

3.1. Effect of N Fertilization Dose, Trichoderma Application, and Mulching on Marketable Yield and Growth Parameters

Marketable yield was significantly affected only by nitrogen fertilization and mulching films; the interaction between the three experimental factors (mulching film; *Trichoderma* spp., nitrogen fertilization) was not detected. The nitrogen dose boosted the marketable yield that increased at higher nitrogen levels but without significant differences between the N60 and N90 treatments (Figure 1). Among the two mulching films, the photoselective film elicited marketable yield, with a 7.9% increase over PE.

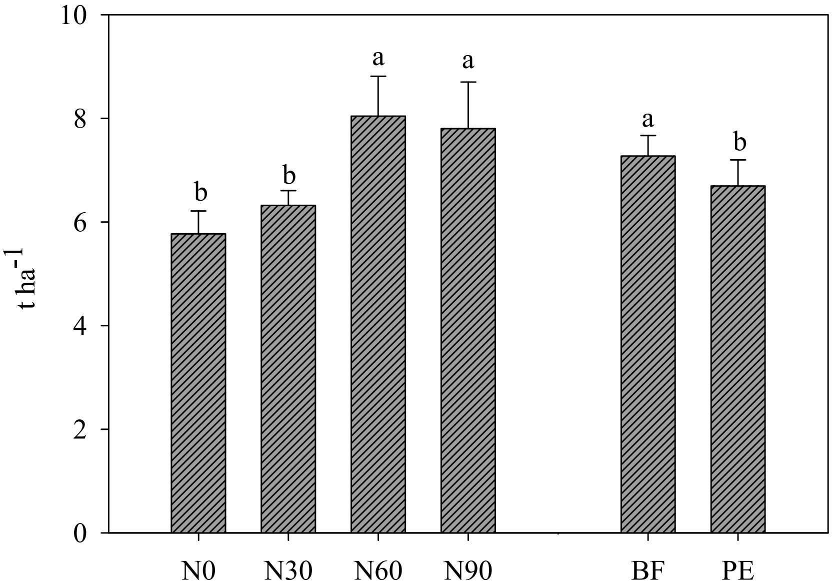

Figure 1. Lettuce yield in relation to mulching cover films (brown photoselective film—BF and black polyethylene—PE) and N fertilization rates (N0 = 0, N30 = 30, N60 = 60, and N90 = 90 kg ha^{-1}). Yield is presented as mean values of three replicates with relative standard errors. Different letters indicate significant differences at $p \leq 0.05$. All data are expressed as mean; n = 3.

In a perspective of sustainable agriculture, the scientific community is evaluating the possibility of using eco-friendly products, such as microbial biostimulants, including *Trichoderma* based product, to reduce undesirable footprint on the environment, due to an excessive or incorrect use of chemical means. In this study, *Trichoderma* did not have a positive effect on marketable yield, contrarily to the results of Fiorentino et al. [22] and Caruso et al. [20]. However, Fiorentino et al. [22] also found that there was no effect on rocket production, when treated by different *Trichoderma*-based biostimulants These results demonstrate that the response to *Trichoderma*-based products could be species-specific, particularly related to the different duration of crop cycles. In fact, we suppose that the effect of RYZO PEP UP on marketable yield was not significant, which was probably due to the lack of sufficient time for the fungi to establish in the soil/roots, boost the colonies and thus their activity. In effect, Chang et al. [29] also found a positive correlation between the colonies of *Trichoderma* in the soil and dry matter content in cucumber; therefore, we suppose that *Trichoderma* might need more time for fully colonizing the soil/roots zone. Instead, the recorded yield increase in lettuce grown on brown film could be due to specific thermal properties of this film. In fact, Guerrini et al. [30] found that the brown mulching film has very good thermal properties, which can guarantee higher temperatures (about +2–3 °C) than black film at the level of root, particularly at 5 to 10 cm depth, where the root system of lettuce is concentrated. This technical characteristic offers many agronomic benefits, especially for winter transplants, since the environmental conditions for plants are better; moreover, it limits the stress due to low temperatures and helps the plant to better form its root system. In a recent study, Bonanomi et al. [31] compared the effect of photoselective mulching film (yellow) to conventional black film, in order to verify their effects, both alone and combined with microbial consortia, on the yield of several crops including winter lettuce, where they found that the yellow photoselective film increased crop yield.

Regarding growth parameters (fresh weight, diameter, and leaf number), only the main effect of nitrogen fertilization and mulching film was found (Table 1). The photoselective mulching film improved all growth parameters, with 7.9%, 5.2%, and 8.8% for head fresh weight and diameter, and head leaf number, respectively. These growth parameters also increased gradually when N fertilization levels increased from 0 to 60 kg ha^{-1}, which showed +28.3%, +10.3%, and +15.0% over no fertilized plants (N0) for fresh weight, head diameter, and leaf number, respectively; instead, there were no significant differences among the N60 and N90 treatments (Table 1).

Table 1. Lettuce growth parameters in relation to mulching cover films (brown photoselective film —YF and a black polyethylene—PE), *Trichoderma* application (untreated—Control and treated—TR) and N fertilization rates (N0 = 0, N30 = 30, N60 = 60, and N90 = 90 kg ha^{-1}).

Treatments	Fresh Weight (g Plant^{-1})	Head Diameter (cm)	Number of Leaves (No. Plant^{-1})
Mulching			
BF	290.83 a	25.22 a	47.75 a
PE	267.90 b	23.89 b	43.54 b
Fertilization			
N0	230.80 c	23.15 b	41.50 c
N30	252.79 b	23.86 b	44.42 b
N60	321.74 a	25.82 a	48.83 a
N90	312.12 a	25.39 a	47.83 a
Trichoderma			
Control	281.65	24.30	46.04
TR	277.07	24.81	45.25
Significance			
Mulching (M)	**	*	*
Fertilization (F)	**	**	*
Trichoderma (TR)	NS	NS	NS
M × F	NS	NS	NS
M × TR	NS	NS	NS
F × TR	NS	NS	NS
M × F × TR	NS	NS	NS

NS, *, ** Non-significant or significant at $p \leq 0.05$ and 0.01. Different letters within each column indicate significant differences according to Duncan's test ($p \leq 0.05$). All data are expressed as mean; n = 3.

3.2. Effect of N Fertilization Dose, Trichoderma Application, and Mulching Films on Leaf Colorimetry and SPAD Index

The SPAD index is a key indicator of the nutritional status of plants, and its measurement is rapid and non-destructive. In this research, it was significantly affected by mulching films and nitrogen fertilization (Table 2). Particularly, the brown film slightly elicited the SPAD index compared to polyethylene film. Moreover, the SPAD index gradually increased when the nitrogen dose increased, but without differences between N60 and N90 treatments (Table 2): the average increase of fertilized over non-fertilized plants was 6.3%.

Table 2. Lettuce Soil Plant Analysis Development (SPAD) index and CIELAB color parameters (L *, a *, and b *) in relation to mulching cover films (brown photoselective film—YF and black polyethylene—PE), *Trichoderma* application (untreated—Control and treated—TR) and N fertilization rates (N0 = 0, N30 = 30, N60 = 60, and N90 = 90 kg ha^{-1}). [L* (lightness, ranging from 0 = black to 100 = white), a * [chroma component ranging from green (−60) to red (+60)], b * [chroma component ranging from blue (−60) to yellow (+60)].

Treatments	SPAD	L *	a *	b *
Mulching				
BF	38.62 a	49.89	−11.11	34.56
PE	37.79 b	48.95	−11.66	36.07
Fertilization				
N0	36.36 c	48.02 c	−10.69 a	36.84
N30	38.04 b	49.15 bc	−11.42 ab	35.73
N60	39.50 a	50.68 a	−11.61 bc	34.88
N90	38.93 a	49.83 ab	−11.82 c	33.81
Trichoderma				
Control	38.04	49.55	−11.44	34.90
TR	38.38	49.28	−11.31	35.73
Significance				
Mulching (M)	**	NS	NS	NS
Fertilization (F)	**	*	*	NS
Trichoderma (TR)	NS	NS	NS	NS
M × F	NS	NS	NS	NS
M × TR	NS	NS	NS	NS
F × TR	NS	NS	NS	NS
M × F × TR	NS	NS	NS	NS

NS, *, ** Non-significant or significant at $p \leq 0.05$ and 0.01. Different letters within each column indicate significant differences according to Duncan's test ($p \leq 0.05$). All data are expressed as mean; n = 3.

The product color is probably the main physical property that consumers observe when deciding a purchase. In the present study, the CIELAB parameters was only affected by nitrogen fertilization (Table 2). Interestingly, augmenting nitrogen fertilization from 0 to 90 kg ha^{-1}, the a* value significantly decreased, reflecting the increase of the green intensity, contemporary with the increase of brightness (L *; Table 2).

3.3. Effect of N Fertilization Dose, Trichoderma Application, and Mulching Films on Antioxidant Capacity and Bioactive Content

LAA, HAA, total phenols, and TAA were affected by nitrate fertilization; mulching films affected TAA, and finally, *Trichoderma* application affected HAA and TAA (Table 3). The interaction between the factors was never significant, except for the interaction of mulching films × *Trichoderma* regarding LAA (Figure 2) and HAA (Figure 3).

The antioxidant activity is one of the most important aspects in determining the nutritional quality of many foods, green leafy vegetables among them. The antioxidant molecules, such as ascorbic acid and phenols, have beneficial effects on human health, because they play a key role in delaying oxidative damage; therefore, they prevent several diseases [32,33].

The photoselective film enhanced total ascorbic acid; instead, there were no detected differences for the other parameters. LAA and HAA antioxidant activities, total phenols, and TAA showed a decreasing trend when nitrogen fertilization doses increased (Table 3). These results are in agreement with the results of Fiorentino et al. [22], who found a decrease in TAA values in lettuce leaves when N doses increased, and they are also in line with the findings of Di Mola et. [34], who found that HAA and TAA in baby lettuce decreased at high nitrogen application. Finally, Wang et al. [35] also observed a reduction of fruit and leafy vegetables quality (soluble solids and ascorbic acid) at high nitrogen fertilization doses. Probably, this behavior is due to the fact that the plants produce antioxidant compounds in response to stress; therefore, when nutritional condition is optimal, the plants show a lower antioxidant activity. Interestingly, *Trichoderma* improved HAA and TAA with an increase of 11.2% and 5.9%, respectively (Table 3). These findings are consistent with the results of Lombardi et al. [36], who applied three different *Trichoderma* bioactive metabolites on strawberry and found that one of them (HYTLO1) promoted the accumulation of ascorbic acid. Similarly, Rouphael et al. [21] and Caruso et al. [20] demonstrated an increase in TAA when *Trichoderma* was applied to lettuce and rocket, respectively. Nonetheless, HAA was as well higher in rocket treated with *Trichoderma* [20].

Table 3. Lipophilic (LAA) and hydrophilic (HAA) antioxidant activities, total phenols, and total ascorbic acid (TAA) in relation to mulching cover films (brown photoselective film–BF and black polyethylene–PE), Trichoderma application (untreated—Control and treated—TR) and N fertilization rates (N0 = 0, N30 = 30, N60 = 60, and N90 = 90 kg ha^{-1}).

Treatments	LAA (mM Trolox 100g^{-1} dw)	HAA (mM AA 100g^{-1} dw)	Phenols (mg gallic acid g^{-1} dw)	TAA (mg g^{-1} fw)
Mulching				
BF	9.52	6.69	1.552	19.59 a
PE	10.42	7.16	1.674	18.16 b
Fertilization				
N0	11.75 a	7.68 a	1.723 a	21.98 a
N30	10.70 ab	7.62 a	1.704 ab	20.76 b
N60	8.97 ab	6.14 b	1.576 bc	18.30 c
N90	8.48 b	6.24 b	1.448 c	14.47 d
Trichoderma				
Control	9.70	6.51 b	1.676	18.30 b
TR	10.24	7.33 a	1.549	19.45 a
Significance				
Mulching (M)	NS	NS	NS	*
Fertilization (F)	*	**	*	**
Trichoderma (T)	NS	*	NS	*
M × F	NS	NS	NS	NS
M × T	*	*	NS	NS
F × T	NS	NS	NS	NS
M × F × T	NS	NS	NS	NS

NS, *, ** Non-significant or significant at $p \leq 0.05$ and 0.01. Different letters within each column indicate significant differences according to Duncan's test ($p \leq 0.05$). All data are expressed as mean; n = 3.

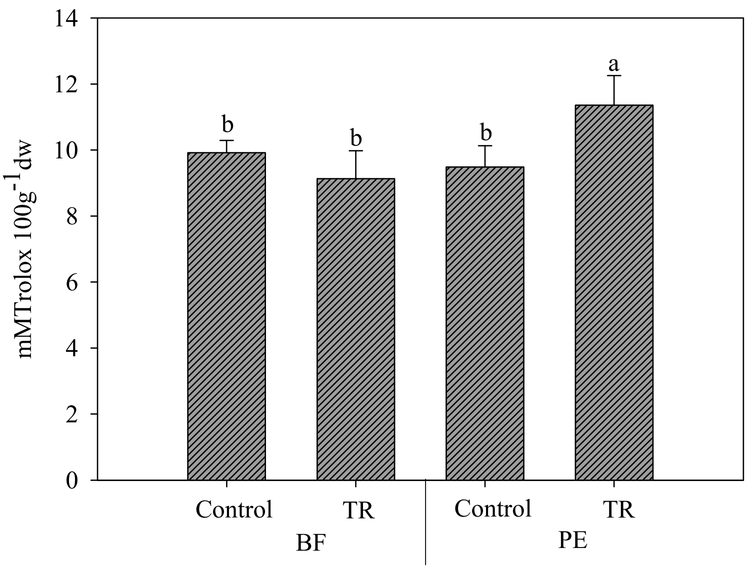

Figure 2. Effect of mulching films (brown photoselective film—BF and black polyethylene—PE) and *Trichoderma* application (untreated—Control and treated—TR) on lipophilic (LAA) antioxidant activity of lettuce leaves. All data are expressed as mean; n = 3.

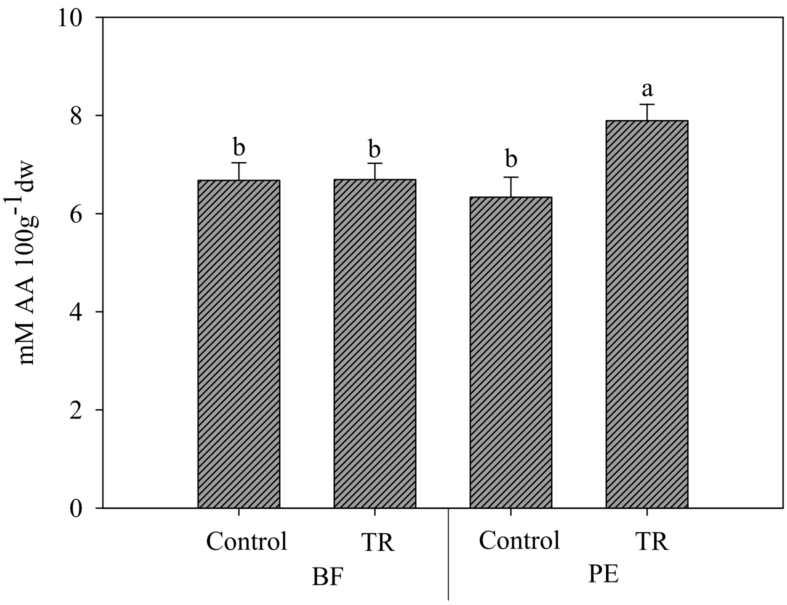

Figure 3. Effect of mulching films (brown photoselective film (BF) and black polyethylene (PE)) and *Trichoderma* application (untreated—Control and treated—TR) on hydrophilic (HAA) antioxidant activity of lettuce leaves. All data are expressed as mean; n = 3.

Regarding lipophilic and hydrophilic antioxidant activities, the combined effects of mulching film and application of *Trichoderma* were detected (Figures 2 and 3, respectively). Interestingly, for both qualitative parameters, the plants treated with *Trichoderma* and grown on black polyethylene showed the best performance, while the other three treatments were not different among them. In particular, the increase was 16.3% and 16.8% for LAA and HAA, respectively. Several studies report that *Trichoderma* grows best in a temperature range of 25 to 30 °C [37,38], and Guerrini et al. [30] found that black film reaches higher temperature in the soil–film gap, compared to other films (brown, transparent, and yellow film), since the surface of the black film heats up more than other films, and the heat is transferred directly to the contact layer. Therefore, since we sprayed *Trichoderma* solution exactly on the soil surface, we suppose that in this zone, the temperature conditions were better for the *Trichoderma* development and activity, and this probably boosted the antioxidant activity of lettuce.

3.4. Effect of N Fertilization Dose, Trichoderma Application, and Mulching Films on Biochemical Parameters and Nitrate Content

All parameters (chlorophyll a, b, total carotenoids and nitrate content) were affected by nitrogen fertilization doses. Moreover, chlorophyll b and nitrate content were also affected by *Trichoderma* application and total chlorophyll by mulching films (Table 4). Particularly, black PE enhanced chlorophyll (a, b and total), carotenoids, and nitrate content, but it was significant only for total chlorophyll, with an increase of 7.7% over photoselective film. Nitrogen fertilization positively affected the chlorophyll and carotenoids content but without significant differences among the nitrogen treatments. Chlorophyll a, b, total chlorophyll, and carotenoids increased on average +19.5%, 24.6%, 21.1%, and 15.8% compared to unfertilized plants, respectively (Table 4). Di Mola et al. [2,9] found a similar trend in baby lettuce and baby rocket regarding these parameters. However, the nitrogen fertilization negatively affected nitrate content in leaves, which increased from 0 to 60 kg N ha^{-1}, but without differences between N60 and N90. The increase in nitrate content is consistent with previous findings on baby leaf lettuce, baby rocket, lamb's lettuce, and spinach [9,16,34]. Finally, the effect of *Trichoderma* was evident on chlorophyll b (+12.8% over the control) and on leaves nitrate content, which was 8% more than control plants; however, this value was under the limit imposed by the European Union (EU) for lettuce marketing (Commission Regulation No. 1258/2011; 3000 to 5000 mg NO_3^- kg^{-1}

of lettuce depending on growing season and cultivation conditions) [39]. The highest value of nitrate content is probably due to an easy uptake determined by *Trichoderma*, which also is able to solubilize Fe_2O_3, CuO, and metallic Zn, via (1) acidification by organic acids, (2) chelation by siderophores, (3) redox by ferric reductase, and (4) hydrolysis by phytase [40].

Table 4. Chlorophyll (a, b, and total), carotenoids, and nitrate content in relation to mulching cover films (brown photoselective film—YF and black polyethylene—PE), *Trichoderma* application (untreated—Control and treated—TR) and N fertilization rates (N0 = 0, N30 = 30, N60 = 60, and N90 = 90 kg ha^{-1}).

Treatments	Chlorophyll a (mg g^{-1} fw)	Chlorophyll b (mg g^{-1} fw)	Total Chlorophyll (mg g^{-1} fw)	Carotenoids (µg g^{-1} fw)	Nitrate (mg g^{-1} fw)
Mulching					
BF	0.552	0.224	0.776 b	293	1570.7
PE	0.599	0.242	0.841 a	309	1526.7
Fertilization					
N0	0.487 b	0.187 b	0.674 b	264 b	1163.8 c
N30	0.591 a	0.246 a	0.838 a	301 ab	1479.9 b
N60	0.606 a	0.246 a	0.852 a	316 ab	1794.3 a
N90	0.618 a	0.252 a	0.871 a	324 a	1756.7 a
Trichoderma					
Control	0.581	0.217 b	0.798	311	1483.9 b
TR	0.570	0.249 a	0.819	292	1613.5 a
Significance					
Mulching	NS	NS	*	NS	NS
Fertilization	*	*	*	*	**
Trichoderma	NS	*	NS	NS	*
M × F	NS	NS	NS	NS	NS
M × T	NS	NS	NS	NS	NS
F × T	NS	NS	NS	NS	NS
M × F × T	NS	NS	NS	NS	NS

NS, *, ** Non-significant or significant at $p \leq 0.05$ and 0.01. Different letters within each column indicate significant differences according to Duncan's test ($p \leq 0.05$). All data are expressed as mean; n = 3.

4. Conclusions

The photoselective film improved the marketable yield and growth parameters of lettuce (head weight, diameter, and leaf number), as well as the nitrogen fertilization (30 and 60 kg ha^{-1}). Moreover, nitrogen also enhanced the SPAD index, color parameters (brightness and green intensity), chlorophyll, and carotenoids content. The increasing levels of nitrogen also determined an increase in nitrate content in leaves but without overcoming the limit imposed by the European community. The application of *Trichoderma* had no positive effects on marketable yield and growth parameters, but it had an encouraging effect on antioxidant activity, especially when combined with polyethylene mulching film. Although the *Trichoderma* had no marked effect on yield, its positive effect on quality traits is an interesting starting point for further research. Its capacity to elicit a higher N uptake could improve production on long cycle-crops, simultaneously allowing a reduction of nitrogen application and mitigating environmental impact.

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Abbreviations

SPAD	Soil Plant Analysis Development
LAA	Lipophilic Antioxidant Activity
HAA	Hydrophilic Antioxidant Activity
TAA	Total Ascorbic Acid

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