Trichoderma-BASED BIO-STIMULANT APPLIED ON OKRA (*Abelmoschus esculentus* L.) BIOPRODUCT APPLIED ON OKRA

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Abstract:

Okra is a popular lucrative crop in African countries but it hasn't yet been scientifically experimented with Algerian open fields. This work aims to examine the potential of a *Trichoderma*-based bio-stimulant on the performance of this crop. The experiment was conducted, in the region of Touggourt, according to a randomized complete block design with three rehearsals. Three plots were treated with the bioproduct (20g/2.5 m^2) and three others were untreated (controls). The assessment was conducted by measuring 13 agro-morphological plant characters associated with growth and yield. The study revealed that the biological product has a stimulating efficiency towards the mostly examined parameters especially the yield, which was doubled compared with the untreated crop. Statistical analysis revealed a significant stimulant effect on quantitative variables of growth (Wilk's Λ= 0.440, F (7, 52) 9.45, p= < 0.05) and yield (Wilk's Λ= 0.72, F (3, 38) 4.89, p= 0.006). Moreover, three stages of development (lifting, flowering, and fruiting) were improved after treatment application. Pearson's Chi^2 test showed that qualitative parameters were strongly associated with the bio-stimulant effect, χ^2= 97, df= 4, p= < 0.05.This treatment incorporated upstream of the culture was very efficient in promoting okra attitude and it can turn local ideology to use organic products in agriculture.

Keywords: Biological product; antagonistic fungus; Agro-morphological character; Organic agriculture; Algeria.

Introduction

Okra (*Abelmoschus esculentus* L.) is a vegetable of high nutritional value. It is rich in minerals, vitamins, and polysaccharides, which are represented in mucilage (Sawadogo et al. 2009; Adetuyi et al. 2012). As well as, fruits of okra have a higher protein efficiency ratio than that of soybeans (Adetuyi et al. 2012). In 2018, plant production was mostly concentrated in tropical and subtropical zones of which India constitutes the main producer (FAO 2020). Moreover, this product was very common in central and western African countries where it constitutes a very lucrative vegetable for low-income communities because of its high market value (Konate et al. 2016; Faostat 2020). There, the botanical species werevery resourceful because of their different expendable organs that were consumed raw, cooked, and

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sometimes-dehydrated (Gemede et al. 2014).

Okra in Algeria, Guenawia as a vernacular name, is especially cultivated in the Southeast of the country; a zone that perfectly meets its soil and climate demands, among other things, the high temperatures (Ranga 2019). Besides, this plant can make a worthy yield if it is reasonably fertilized. In this context, almost all cultures in Algeria concerning regard to okra are submerged in a conventional model, which allows using many chemical compounds, from the seedbed to harvest. This can be justified by a huge lack of bio-alternatives that makes Algeria lagging in the organic farming field, despite the global political incitements taken for decades. Therefore, it is necessary to push research towards the creation of bioproducts to ensure safe and sustainable agriculture.

Trichoderma products were always considered the most effective treatments (Fernando et al. 2018).They were mainly encountered (with different formulations) in Asia, Europe as well as in Central and North America (Woo et al. 2014). Generally, either *Trichoderma* or its products were reported as stimulators of root and shoot systems of several plant species such as cucumber (Yedidia et al. 2001), melon (Fernando et al. 2018), tomato (Bal and Altintas 2006), and bean (Hoyos-Carvajal et al. 2009). Therefore, adding biological agents to substrates using *Trichoderma*-based bioproducts constitutes an alternative technique to chemical amendments and enhances the biological processes of soil and plant physiological functions. This ensures as well plant immunity, qualitative tasty products, and healthy yields (Des fontaines et al. 2018).

For these purposes, we did isolate the indigenous strains of *Trichoderma* from the nearest agro-ecological region where it would be then applied. The potential of this isolate was justified through several *in vitro* studies. That followed this study that was headed by innovating a viable and safe bioproduct powder with stimulating interest and examining its *in vivo* capacity to improve agro-morphological performances of okra.

Materials and methods

Conduct of the essay

The current work was carried out, in the region of Touggourt, at the experimental station of the National Institute of Agronomic Research of Algeria (INRAA) located in Southeastern Algeria, 660 km from the capital city, at geographic points of 33° 04'13" North latitude and 06° 05' 49" East longitude with an altitude of 85 m. Soil texture is sandy to sandy loam with alkaline pH (8.13) and a poor level of organic matter. According to Emberger's classification, the local climate is Saharan with dry Summer and mild Winter. Also, annual precipitations are low and cannot exceed 45 mm over ten years (2008 to 2018).

Okra seeds used in this experiment are derived from a local cultivar that belongs to a local farmer. It is short light green fruit with a pentagonal capsule section and an 80 to 120-day vegetative cycle.

The experimental device of okra was organized in six elementary plots (spaced 1 m apart). A block has two plots (control and experimental) with a surface of 2.5 m^2. In each plot, 25 seeds were cultivated at 40 cm between lines and plants, and fertilization with organic manure was applied at a rate of 40 tons/ha.

Soil preparation

The bio-stimulant used in this work was produced as a part of a mixed research teams project between the institute of INRAA, DGRSDT (Directorate-General for Scientific Research and Technological Development) and the social-economic partner SINAL Company of Oran. The powder product was made by indigenous strains of *Trichoderma harzianum* isolated from soil (strain being better adapted to high temperatures and salinity as well as to the low humidity of the region). The scientific and technical research center in Physico-chemical analysis (CRAPC) analyzed the product and concluded that it is constituted of56 % of linoleic acid, nature mainly organic with 1.8 % of mineral elements and it has a good stability of organic compounds.

The product (10^6 CFU/g) was intended to improve okra development because it proved its efficiency in promoting the growth and yield of safflower in the same region (Lakhdari et al. 2020). Treated plots received a dose of 20g/plot. The incorporation into the soil was realized, 15 days before seeding, by a uniform manual spreading. This interval of time guaranteed a good establishment of beneficial microorganism communities introduced upstream of culture.

Measurements of agro-morphological variables

Okra behavior under bio-stimulant effect was studied by the evaluation of 13 quantitative and qualitative agro-morphological parameters. After 25 days of seedling, 30 randomly selected plants of each treatment were chosen to measure the fresh and dry biomass. Drying was carried out as described in Fondio et al. (2003).On the other hand, biometric variables i.e., length of the plant (Lp), the diameter of the stem (Ds), number of leaves/ plant (Nl), number of branches/ plant (Nb), were examined. These measurements were evaluated, on the last days before harvest, based on a mean of a representative sample of 30 plants/treatment. This was applied as well at yield parameters as yield of fruits/plant (Yf) and several fruits/plant (Nf). Moreover, the length (Lf) and diameter (Df) of three first harvested fruits of some selected plants were measured on a sample of 21 fruits/treatment. This concerns young fruits of two/or three days old. Water contents inplants (Wcp) and fruits (Wcf) were determined according to Ouoba Kondia et al. (2010).

In addition, qualitative data were collected from the entire cultivated area. They were represented by seedlings lifting after 25 days, flowering noted at 50% from appearance, and fruiting at 50 % from appearance.

Exploitation of collected results

Quantitative data were subjected to a multivariate analysis of variance (MANOVA), at a significance level of $p \leq 0.05$, using the SPSS statistic program (version 20.0.). Moreover, categorical variables were treated, according to a Chi-Square test of Independence (χ^2), on a total of 58 plants. Independence of the relationship between both groups was tested on three levels (lifting, flowering, and fruiting).

Results

Compared to those of control, values of recorded means of measured parameters on treated plants showed that the bio-stimulant improved clearly all the studied aspects of okra (Figure1). This was confirmed by MANOVA analysis where there was a significant difference between studied groups, Wilk's $\Lambda = 0.440$, $F (7, 52) = 9.45$, $p= 0.000$, partial $\eta^2 = 0.56$. Separates analyzes of ANOVA ($p = 0.05$), conducted on each dependent variable, showed a significant difference between experimental and control samples where Lp: $F (1, 58) = 54.74$, $p = 0.000$, partial $\eta^2 = 0.49$; Ds: $F (1, 58) = 26.22$, $p = 0.000$, partial $\eta^2 = 0.31$; Nb: $F (1, 58) = 31.19$, $p = 0.000$, partial $\eta^2 = 0.35$; Nl: $F (1, 58) = 24.31$, $p = 0.000$, partial $\eta^2 = 0.30$ and Nf: $F (1, 58) = 19.60$, $p = 0.000$, partial $\eta^2 = 0.25$. Likewise, Yf showed a significant difference between groups $F (1, 58) = 26.80$, $p = 0.000$, partial $\eta^2 = 0.32$.

This product improved also Wc_p and Wc_f of treated okra (Figure1). However, there was no significant difference between studying groups in terms of this quantitative character, $F (1, 58) = 2.41$, $p = 0.126$, partial $\eta^2 = 0.04$. As far as yield parameters, the bioproduct proved again its effect Wilk's $\Lambda = 0.72$, $F (3, 38) = 4.89$, $p = 0.006$, partial $\eta^2 = 0.28$. Subsequent ANOVAs, at ($p = 0.05$), of each dependent variable explained that the significant difference truly concerns Df: $F (1, 40) = 14.47$, $p = < 0.05$, partial $\eta^2 = 0.27$ whereas the bio-stimulant didn't have a significant effect on Lf: $F (1, 40) = 0.02$, $p = 0.88$, partial $\eta^2 = 0.001$ and Wcf: $F (1, 40) = 2.26$, $p = 0.14$, partial $\eta^2 = 0.05$. Moreover, Pearson's Chi-squared test applied on categorical measures of okra revealed a very low probability. On the other hand, there was a significant real effect of the applied bio-stimulant on qualitative parameters, $\chi^2 = 97$, $df = 4$, $p = < 0.05$.

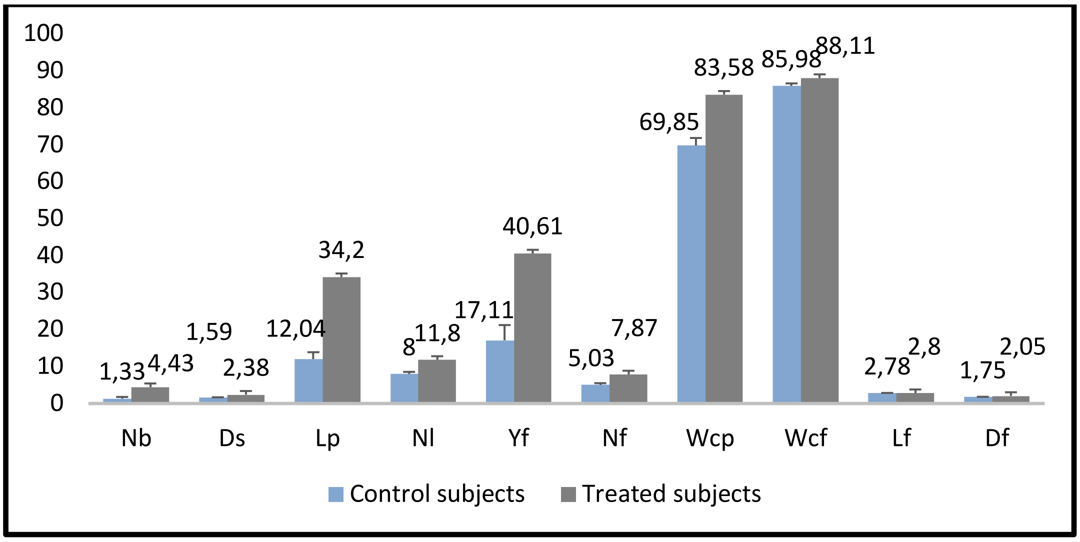

Fig. 1 - Studied quantitative characters of okra under different treatments

(Nb): Number of branches/plants; (Ds) Diameter of the stem; (Lp): Length of the plant; (Nl): Number of leaves/plants, (Yf): yield of fruits/ plant; (Nf): number of fruits/plant, Wc_p: Water content in plants; Wc_f: Water content in flowers; (Lf): Length of fruit; (Df): Diameter of fruit

Discussion

For comparative studies, there were no sufficient works that highlight the effect of *Trichoderma* on okra. Therefore, this is the first paper confirming the potential of the *Trichoderma*-based product in stimulating the growth and the yield of this plant in Algeria as it did on safflower (Lakhdari et al. 2020). As it is the first scientific work carried out on okra in Algeria. Several researchers mentioned that the application of *Trichoderma* improves the growth and yield of several foods and ornamental crops such as cucumber, tomato, melon, and cacao without negative effects on fruit quality (Yedidia et al. 2001; Bal and Altintas 2006; Bae et al. 2009; Fernando et al. 2018). The obtained results conform with those reported by (Fernando et al. 2018). The authors confirmed that the application (irrigation or mixing) of *Trichoderma* on the substrates increased most of the studied growth parameters of melon. In their work carried out on tomatoes, Bal and Altintas (2006) noted that the application of *Trichoderma* (10^8 CFU/g) provides better yields unlike, untreated plants. The same researchers specified that improvements do especially touch the weight and diameter of fruits.

Practically, *Trichoderma* was known as the most important biological Phyto-stimulating agent due to its promotion of the general metabolism of many plant species. This power involves multi-level communication with plants and induces a release of several growth regulators metabolites in the rhizosphere (Lopez-Bucio et al. 2015). For instance, increasing levels of indole acetic acid (IAA) and gibberellic acid, hormones that promote plant root growth, were detected after the application of *Trichoderma harzianum* (Hoyos-

Carvajal et al. 2009; Ortíz-Castro et al. 2009; Sofo et al. 2011; Mukherjee et al. 2013). Therewith, Stewart and Hill (2014) suggested a possible link that may occur between root growth and the production of ethylene, indole acetic acid as well as gibberellic acid. Moreover, *Trichoderma* released volatile organic compounds in ecosystems to allow plants to communicate with neighbors as well as activate their immunity and morphogenesis (Lopez-Bucio et al. 2015; Ortíz-Castro et al. 2009). Indeed, Hung et al. (2013) demonstrated that exposure of *Arabidopsis thaliana* plants to volatile compounds released by *Trichoderma viride* promotes growth, fresh weight, root mass, and chlorophyll concentration in plant leaves.

This fungus helps plants to overcome different types of biotic and abiotic stress through the induction of resistance/tolerance of organisms. Lopez-Bucio et al. (2015), Singh et al. (2011), as well as Li-Marchetti and Joussemet (2014), reported that *Trichoderma* enhances the genes expression of plants to better resist biotic and abiotic constraints. The potential of *Trichoderma* was expressed in overcoming various abiotic disorders such as water, salt, and thermal stress exerted on corn, tomatoes, and cocoa(Bae et al. 2009; Harman 2000; Mastouri et al. 2010). The same authors reported that this efficiency was represented in the promotion of deepening, weight, and density of roots that then lead to better water gaining. Besides, another study mentioned that the early addition of *Trichoderma* to substrate and roots improves the assimilation of nitrogen (Adetuyi et al. 2012). Mahato et al. (2018) as well found that supporting *Trichoderma* with a minimized dose of nitrogen fertilizer (NPK) and organic manure improves most of the growth and yield of wheat. Shoresh et al. (2010) were further reported that *Trichoderma* reduces, by 40%, the use of nitrogen fertilizer on corn. Therewith, (Yedidia et al. 2001) reported that *Trichoderma* makes phosphorus and other minerals more accessible to plants due to its mechanisms of acidifying the environment by producing organic acids such as gluconic, citric, and fumaric acids. Here we demonstrate the efficiency of this bio-stimulant in promoting the agro-morphological response of okra in open field conditions. However, studying the infiltration of this bioproduct into the rhizosphere will help to select the plants that can go with it.

Conclusion

View the massive use of chemicals and soil degradation; it is opportune to focus on long-term management goals that improve yield quantity and quality without collateral damages to the environment. *Trichoderma*-based bio-stimulant delivered improvements on okra, at a different stage of plant development (seedlings, growth, flowering, and fruiting precocity, and yield) that demonstrate new sustainable agriculture in Algeria. It is just preferable to inoculate this bioproduct in substrates, upstream of the crop, with the restrictions already described to ensure better colonization of the rhizosphere and maintain synergistic positive effects with other agronomic practices.

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Conflict of interest: None

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