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Original Research Article

Effects of Mycorrhizae and Aqueous Extracts of *Hyptis suaveolens* and *Cyperus rotundus* on Cotton Productivity in the Far North Region (Cameroon)

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Abstract: Cotton cultivation in Cameroun is characterized by low yields due to soil infertility and pest infestation. Sustainable improvement requires the use of mycorrhizae combined with aqueous extracts from *Hyptis suaveolens* and *Cyperus rotundus* rhizomes. The experimental design used is 7×2×2 split-plot with to factors and seven treatments: (control (Te-); chemical input (Te+); H. *suaveolens* (H); C. *rotundus* (C); mycorrhizae (M); two combinations: mycorrhizae-H. *suaveolens* (M+H) and mycorrhizae-C. *rotundus* (M+C); two varieties (Q302 and L457) and two localities (Kodek and Mouda). The treatments M+H, M+C, mycorrhizae (M), aqueous extracts of H. *suaveolens* and C. *rotundus* (C) improved cotton yield by 30,91; 29,66; 26,34; 9,96 and 7,01 % respectively compared to the Te- treatment. Similarly, the Te+, M+H, M+C, mycorrhizae (M), H. *suaveolens* extract (H) and C. *rotundus* extract (C) treatment improved cotton seed yield by 26,14; 26,67; 22,25; 18,04; 14,84 and 10,08 % compared to the Te- treatment. Overall, there were no significant differences between plots treated with the combination of mycorrhizae and aqueous extracts of H. *suaveolens* or C. *rotundus* could be an alternative to the use of chemical inputs in order to improve cotton productivity in the Far North Region of Cameroon while ensuring sustainable agriculture.
Keywords: Cotton, Productivity, Aqueous Extracts, Mycorrhizae, Cameroon.

INTRODUCTION

The cotton plant (*Gossypium hirsutum* L.) is a shrub belonging to the Malvaceae family, growing to a height of between 1 and 1,5 m. this plant plays an important role worldwide (Folefack, 2010), producing a fiber called cotton that is used to weave clothing (SODECOTON, 2015). Cotton cultivation also produces various derivatives that play an important role in human and livestock nutrition. The seeds obtained from ginning are used either as seed or in oil production.

In addition, the seed kernel has a protein content of 20 to 30 % (FAO, 2014). In Africa, its cultivation is an important source of foreign trade revenue and a vital source of income for more than 10 million people (CTA, 2005). Approximately 2 million farmers produce an average of more than 2 million tons of cottonseed per year, or nearly 830. 000 tons of fiber at an average ginning yield of 41, 5 %. This volume of cotton fiber represents more than 15% of international trade, with a turnover ranging between 500 and 700 billion CFA francs (CMA/AOC, 2017).

In Cameroon, cotton cultivation covers an area of 85.000 Km^2 and involves around 200.000 producers, with an annual production of 250.000 tons of cottonseed (SODECOTON, 2018). More than two million people depend in one way or another on the cotton value chain (financial income, consumption of oil from cotton seeds,

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textile clothing, and livestock feed from oilseed meal) (EIB, 2017). Despite its multiple uses, cotton cultivation unfortunately faces several challenges in Cameroon, including rising input costs (chemical fertilizers, pesticides, etc.), declining soil fertility, and increasing pest infestations (Yao, 2011; CMA⁄AOC, 2017). In the absence of phytosanitary protection, cotton pests can cause a loss of more than 45.95% of the crop (SODECOTON, 2018). Cotton producers generally use chemical pesticides to control pests and synthetic chemical fertilizers to improve soil fertility and increase productivity. However, chemical control poses serious environmental risks and can lead to pests developing new resistances (Ouorou *et al*., 2012). In fact, cotton cultivation accounts for only 2.4% of cultivated land but consume 16% of the world's insecticides, posing a great danger to human health and ecosystems (Helvetas *et al.,* 2008). The excessive use of chemical fertilizers and pesticides results in soil degradation, lower yields, pesticide resistance in pests, collateral damage to beneficial insects, water pollution, and a significant reduction in cotton producer's incomes (FAO, 2014). Due to their harmful effects, alternatives must be found (Renou *et al*., 2016).

It is therefore necessary to consider management methods in local farming communities that enable the rational and sustainable exploitation of bioresources (Manlay, 2000) and increase agricultural production while protecting natural ecosystems (Megueni *et al*., 2011). The use of mycorrhizal biofertilisers, aqueous extracts from *Hyptis suaveolens* leaves and *Cyperus rotundus* rhizomes has proven to be a solution for restoring soil fertility on the one hand reducing pest damage on the other. Mycorrizal inoculum for cotton cultivation may help improve the plant's hydromineral nutrition and resistance to pests. Aqueous extracts from H. *suaveolens* leaves and C. *rotundus* rhizomes could be used as phytopesticides. These aqueous extracts offer a real advantage due to their low persistence, low toxicity to humans and their mode of action (repellent or anti-appetitive) on pests (Ngamo *et al.,* 2007). However, no work has been done on the use of mycorrhizae and aqueous extracts from these plants in association with cotton cotton cultivation. This study aims to improve cotton productivity while limiting the use of chemical inputs. Specifically, it will involve: (1) evaluating cotton germination rates; (2) evaluating cotton yields.

MATERIALS AND METHODS

Our research was conducted in the Far North region of Cameroon. Two sites were selected for fieldwork in this region. The first site is located in Kodek, about 15 km from Maroua. Its soil is sandy loam (representing 87% of the soil texture) and small part of its texture is clay (Seignobos *et al*., 2000). The geographical coordinates of this site are: 10° 38'43'" north latitude and 14° 24"27" east longitude, at an altitude of 523 m. These sites were chosen for their cotton farming practices by local farmers. The rainfall pattern is mainly monomodal, with a tendency for precipitation to decrease and temperatures to rise over the years (ONACC, 2018). The climate in the area is Sahelian, with a long dry season lasting nine months (October to june) and a short rainy season lasting three months (july to September). The average annual temperature is 39° C, with daily highs often reaching 45° C in April and May. The average annual rainfall is around 900-1000 mm (CTFC, 2011).

Cotton Varieties Tested

Two cotton varieties, IRMA Q302 and IRMA L457, of the genus Gossypium hirsutum (Malvaceae) were used as plant material. These two varieties were supplied by the Agricultural Research Institute for Development (IRAD) in Far North Cameroon. They were chosen because of their intensive use by farmers in the region (figure 1).

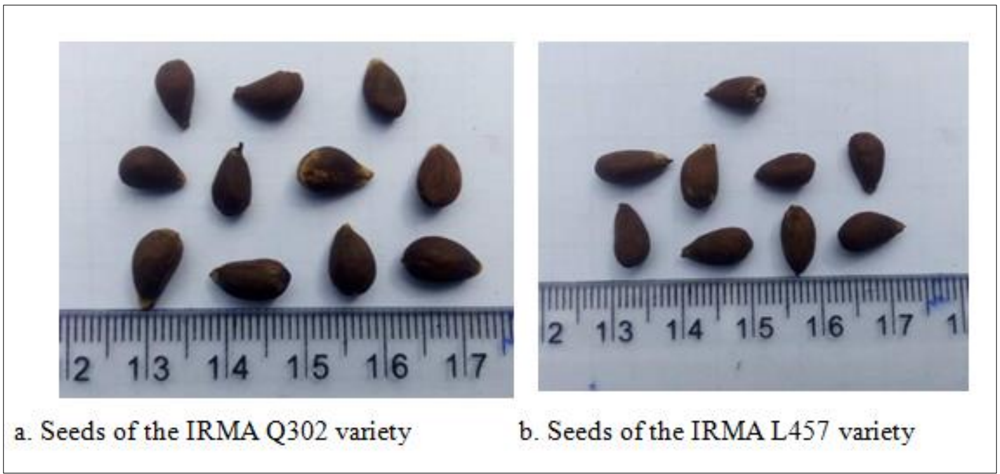

Figure 1: Cotton seeds (Abakar *et al*., 2019)

Aqueous Extracts of *Hyptis Suaveolens* and *Cyperus Rotundus*

Aqueous extracts from the rhizome of Cyperus rotundus (figure 2) and the leaves of Hyptis suaveolens (figure 3) were used as bioinsecticides in this study. To obtain these extracts, the leaves of H. suaveolens and the rhizomes of C. rotundus were harvested and dried in the shade for 24 hours, after which these plant parts were stored in the laboratory for 12 days before being finely ground using an electric grinder (Victoria Grain Mill-High Hopper, Medellin, Colombia). The method used to obtain the aqueous powder extracts is described by Kumar (2003) and Kosma (2011).

Figure 2: Plant, rhizomes, and aqueous extract of *Cyperus rotundus* rhizomes (Abakar *et al*., 2020)

Figure 3: Plant, leaves, and aqueous extract of Hyptis suaveolens leaves (Abakar *et al*., 2020)

The mycorrhizal inoculum used for this work comes from the collection of the Soil Laboratry of the Biotechnology centre at the University of Yaounde I. it consist of mixture of soil (clay, sand grains) and root fragments from plants containing spores of fungi of the genus Glomus and Gigaspora at a concentration of 20 spores⁄g of substrate (figure 4) according to jansa *et al*., (2002), arbusccular mycorrhizal fungi of the genera Glomus spp. and Gigaspora spp. Are the best known. Glomus is an obligate symbiotic fungus that is not very host-specific (Bouamri *et al*., 2006; Wang *et al*., 2008).

Figure 4: Mycorrhizal inoculum (Abakar *et al*., 2019)

The chemical inputs used in this experiment were purchased from the Cotton Development Corporation (SODECOTON) in Maroua. This was a single application of NPKBS complete fertilizer with the formula 21.08.12.3. This type of fertilizer is intendet for cotton cultivation and is commonly used by cotton producers in the Far North (figure 5). This chemical fertilizer was applied fourteen days after sowing. The chemical insecticide used throughout the experiement is commonly used by farmers. Its trade name is trofort 400 EC and its active ingredient is organophosphate combined with pyretroid.

Ingredient is organophosphate combined with pyrethroid.

a. Chemical fertilizers (NPKBS: 21. 08.12.) b. Chemical insecticide (Trofort)

Figure 5: Chemical inputs (Abakar *et al*., 2019)

The experimental design used in each location (Kodek and Mouda) is a two-factor split-plot; the main factor consists of cotton varieties (Q302 and L457 and the secondary factor consist of inputs applied to cotton (chemical fertilizer and chemical insecticide (positive control) Te+); plants that received no treatment (negative control; Te-); aqueous extract of Hyptis suaveolens leaves (H); aqueous extract of Cyperus rotundusrhizomes (C); combination of mycorrhizae and aqueous extracts of *Hyptis suaveolens* leaves (M+H) or *Cyperus rotundus* rhizomes (M+C). Each experimental unit comprises three rows of seedlings, and each row comprises ten pots, giving a total of 30 pots per elementary plot.

The sowing phase took place on June 3 and 5, 2019, in Kodek and Mouda, respectively. The sowing method used was direct seeding. Two to three cotton seeds were sown per hole at a depth of approximately 2 to 3 cm (SODECOTON, 2015). Twenty grams of mycorrhizae were added per hole (Abakar *et al*., 2019). Mycorrhizal inoculation was carried out at sowing, and the inoculum was placed in direct contact with the seeds and throughout the cavity of the hole. To ensure that the plants were well anchored in the soil, NPKSB chemical fertilizer was applied fourteen days after sowing. This chemical fertilizer was applied directly at the base of the seedling (Deschênes, 2010). The field was maintained every two weeks by weeding, thus promoting good nutrition and water absorption.

Insecticide treatments (Trofort and aqueous solution of Hyptis suaveolens and Cyperus rotundus) were carried out on July 24, 2019, i.e., 45 days after sowing. The Trofort insecticide was applied to all aerial parts of the plants. Treatment with aqueous extracts of H. *suaveolens* and C. *rotundus* was carried out every 7 days, for a total of 12 treatments. A Micron rotary disc sprayer was used to apply the insecticides in the field.

Determination of the emergence rate and number of nodes per cotton plant: The emergence rate of the plants was assessed 14 days after sowing. To assess the emergence rate of the plants, the number of plants that emerged was counted in order to evaluate the emergence rate per treatment. The emergence rate was calculated using the method employed by Djossin *et al*., (2013): ER = NEP × 100/TES, where ER = emergence rate, NEP = number of plants that emerged, and TES = total number of seeds sown. The number of nodes on the main stem of the cotton plant was determined by simple counting 80 days after sowing, i.e., at capsule maturity.
TL=NPL ×100/NTP
TL: emergence rate;
NPL: number of plants emerged;
NTP: total number of seeds sown.

The number of nodes on the main stem of the cotton plant is determined by simple counting 80 days after sowing, i.e., when the bolls are mature.

Data Analysis:

The data were statistically analyzed using Stratigraphic Plus version 5.0 software, which performs analysis of variance (ANOVA). Duncan's test was used to assess the difference between the treatment means.

RESULTS AND DISCUSSION

Cotton Emergence Rate

The results for cotton emergnce rate are shown in figures 6 and 7. Analysis of variance indicates that there is a significant difference ($P<0.05$) between treatments in terms of cotton emergence rate. It varies between 48.66±2,43 % for the treatment that received no fertiliser (Te-) of the Irma Q302 variety at the Mouda experimental site and 85.33±4.21 % for the treatment enriched with mycorrhizae of the Irma Q302 variety at the Kodek experimental site. The subplots enriched with mycorrhizae had a high germination rate compared to the plots that received no treatment (Te-). This rate varies between 70.66±3.51 for the Irma Q302 variety in Mouda and 85.33±4.21 for the L457 variety in Kodek. However, there are no significant difference ($p<0.05$) between varieties and study sites in terms of cotton plant emergence rates.

The percentage germination rate obtained differs from that of Djossin (2013), who obtained values between 34.75 and 56.25 % for the cotton germination rate in his study on the response of cotton (Gossypium hirsutum L.) to different doses of urea and sowing densities after improved fallow based on Mucuna pruriens at the Barre site in the commune of Zogbodomey (Abomey). This difference in results could be explained by the different agroecological zones and varieties. On the other hand, the difference in emergence rates between treatments may be due to the protection of cotton seed varieties by mycorrhizae against pathogens, mainly fungi and nematodes that attack plant seeds and roots (Fortin *et al*., 2008; Doidy *et al*., 2012). Indeed, mycorrhization induces an active state of immunisation in the plant, enabling it to respond more effectively to pathogen attacks (Ismail *et al*., 2012; Jung *et al.*, 2012). The mycorrhizae added to the soil promoted the emergence of plants of the two cotton varieties used at the different trial sites.

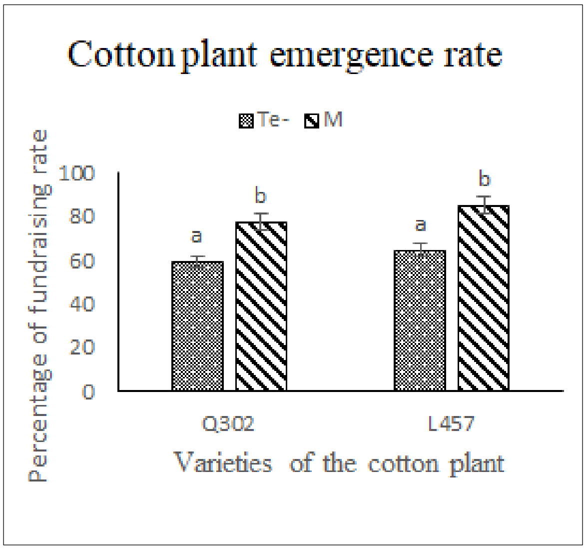

Figure 6: Emergence rate of different cotton varieties at the Kodek site

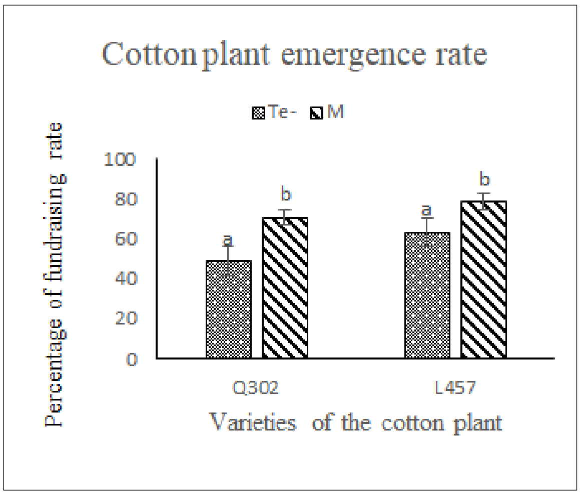

Figure 7: Emergence rate of different cotton varieties at the Mouda site

TE: Plot that received no treatment, **M:** plot enriched with mycorrhizae. The values of bands followed by the same letter in a locality are not significantly different at the 5% threshold.

Number of Nodes on the Main Stem

Figures 8 and 9 show the number of nodes on the main stem according to treatment. Analysis of variance indicates that there is a significant difference ($p<0.05$) between treatments. The results show that the lowest number of nodes on the main stem of the cotton plant was found on plants from unfertilized plots (Te-) with an average of 12.70±3.16 nodes for the Te- treatment of the L457 variety at the Mouda trial site. On the other hand , the highest number of nodes on the main stem was found on plants from the plot enriched with chemical inputs (Te+) of the Irma Q302 variety in the Kodek experimental plot. However, there were no significant difference ($p<0.05$) between plots enriched with chemical inputs and those enriched with mycorrhizae. Overall, plots enriched with mycorrhizae increased the number of nodes on the main stem of cotton plants by 14.62 % and 15.87 % compared to untreated plots in Kodek and Mouda, respectively.

This study shows that the mycorrhizae used favorably increased the number of main stem nodes in both cotton varieties tested, similar to plots treated with chemical inputs. Both the mycorrhizae and the chemical fertilizer applied to the soil had a positive effect on increasing the number of main stem nodes in both cotton varieties tested. However, the number of main stem nodes did not show clear differences between the study sites or between the cotton varieties. The data obtained regarding the number of main stem nodes are identical to those obtained by Sekloka (2006) and Dagbenonbakin *et al*., (2012), who respectively obtained an average of 12 and 23 main stem nodes in their cotton studies.

This correlation is attributed to similar growing conditions and agro-ecological zones. The number of nodes on the main stem indicates healthy plant development. A higher number of nodes means the plant produces more fruiting branches. These fruiting branches bear the cotton bolls; therefore, an increase in fruiting branches would lead to an increase in cotton bolls, thus improving cotton and seed cotton yields.

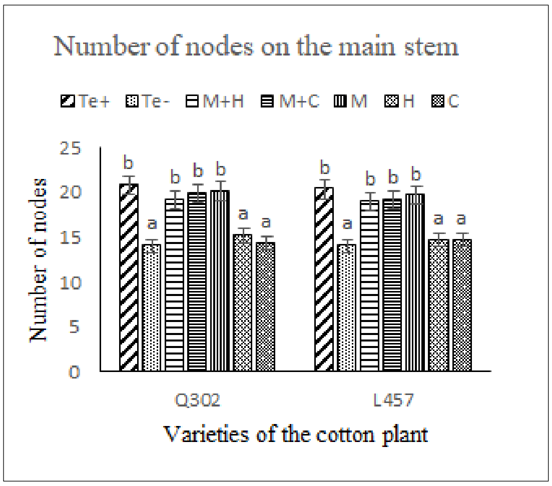

Figure 8: Number of nodes on the main stem of the cotton plant in the locality of Kodek

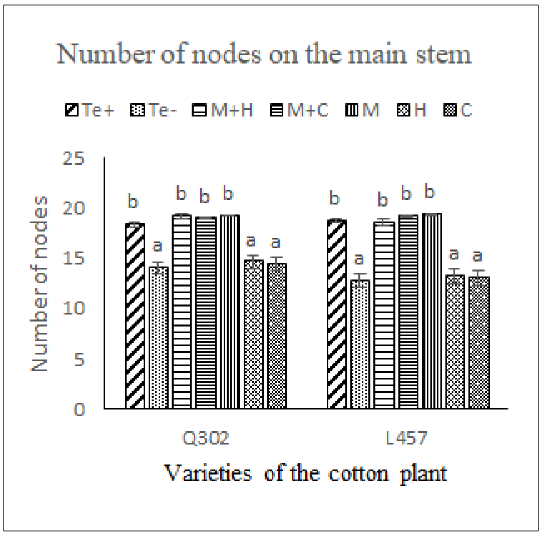

Figure 9: Number of nodes on the main stem of the cotton plant in the locality of Mouda

Cotton Fiber and Seed Cotton Yield

Effects of mycorrhizae and aqueous extracts of *Hyptis suaveolens* and *Cyperus rotundus* on cotton yield.

The results of the effects of mycorrhizae and aqueous extracts of *Hyptis suaveolens* and *Cyperus rotundus* on cotton fiber yield revealed a significant difference (p < 0.05) between the treatments (Figures 10 and 11). The plot that received no fertilizer (Te-) of the Q302 variety at the Kodek experimental site had the lowest cotton yield, with an average of 569.37 ± 12.10 kg/ha. On the other hand, the highest yield was that of the treatment of the combination mycorrhizae aqueous extracts of H. suaveolens (M+H) of the variety L457 in the study site of Mouda with an average of 1286.62±78.45 Kg/ha. owever, there were no significant differences (p<0.05) between the plots treated with the mycorrhizal-aqueous extracts of H. suaveolens and mycorrhizal-aqueous extracts of C. rotundus combinations (M+C) compared to the plot treated with chemical inputs (Te+). This result shows that the M+H, M+C, mycorrhizal (M), aqueous extracts of H. suaveolens (H), and C. rotundus (C) treatments improved yield by 30.91%, 29.66%, 26.34%, 9.96%, and 7.01%, respectively, compared to the Te- treatment.

The results obtained for cotton fiber yield show that the different inputs used reduced the impact of pests on cotton cultivation. This resulted in an increase in cotton yield compared to the Te- treatment. This result corroborates the work of Barbosa *et al*., (2011) and Fall *et al.,* (2017), who respectively studied the toxicity of *Cyperus rotundus* extracts on Diabrotica speciosa and the insecticidal activity of Callistemon viminalis, Melaleuca leucadendron, and *Hyptis suaveolens* essential oils against Sitophilus spp., maize pests. In this regard, they demonstrated that H. *suaveolens* and C. *rotundus* extracts significantly reduce the impact of pests on crops. Thus, this improvement in cotton fiber yield would be due to the effects of extracts from H. *suaveolens* and C. *rotundus* which reduced the impact of pests on the two cotton varieties used. Similarly, mycorrhizae have not only boosted yield but, more importantly, strengthened the plant's natural defense system (Hamza, 2014; Malard, 2016; Lenoir, 2017; and Tobolbai *et al*., 2018). Furthermore, genotypic characteristics should not be overlooked; the L457 variety appears to have genotypic characteristics that are more resistant to pests compared to the Q302 variety. Cotton fiber yield allows us to assess the effects of mycorrhizae and aqueous extracts of H. *suaveolens* and C. *rotundus* on cotton yield. In this study, the combination of mycorrhizae and H. *suaveolens* extracts proved beneficial to cotton fiber yield in the study sites.

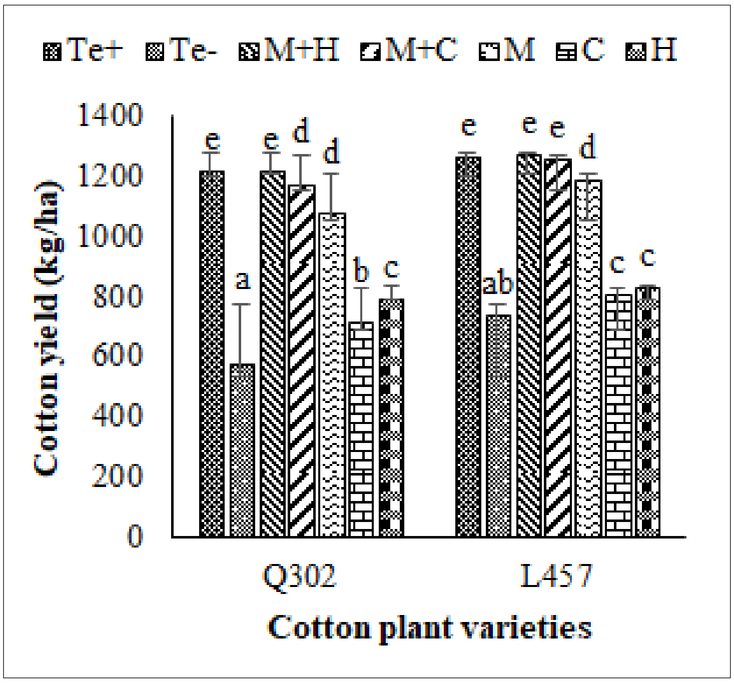

Figure 10: Effects of mycorrhizae and aqueous extracts of *Hyptis suaveolens* and *Cyperus rotundus* on cotton fiber yield in the Kodek locality

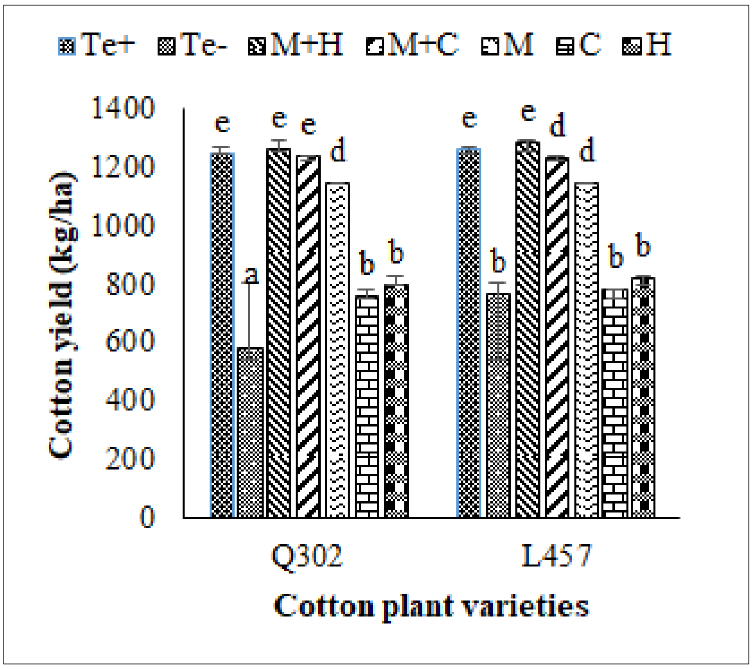

Figure 11: Effects of mycorrhizae and aqueous extracts of *Hyptis suaveolens* and *Cyperus rotundus* on cotton fiber yield in the Mouda locality

Te+: plot treated with chemical fertilizer and chemical insecticide; Te-: plot without inputs; H: plot treated with aqueous extract of H. *suaveolens*; C: plot treated with aqueous extract of C. *rotundus*; M+C: plot combined with mycorrhizae and aqueous extract of C. *rotundus*; M+H: plot combined with mycorrhizae and aqueous extract of H. *suaveolens*; M: plot treated with mycorrhizae.

Effects of Mycorrhizae and Aqueous Extracts of Hyptis Suaveolens and Cyperus Rotundus on Seed Cotton Yield

Statistical analysis of the data on the effect of mycorrhizae and aqueous extracts of *Hyptis suaveolens* and *Cyperus rotundus* on seed cotton yield reveals significant differences ($p < 0.05$) between the treatments (Figures 12 and 13). The lowest seed cotton yield was obtained from the unfertilized plot (Te-) of the Q302 variety in the Kodek locality, with an average of 1012.38 ± 68.13 kg/ha. However, the highest yield came from the plot treated with the combination of mycorrhizae-aqueous extracts of H. *suaveolens* (M+H) of the L457 variety in the Kodek site with an average of 1826.35±51.27 Kg/ha. However, there are no significant differences between the plots treated with the combination of mycorrhizae-extracts of H. *suaveolens* and that of mycorrhizae-aqueous extracts of C. *rotundus* (M+C) compared to the plot treated with chemical inputs (Te+).

Overall, treatments Te+, M+H, M+C, M, H, and C improved seed cotton yield by 26.14%, 26.67%, 22.25%, 18.04%, 14.84%, and 10.08%, respectively, compared to treatment Te-. This result demonstrates that mycorrhizae and extracts of H. *suaveolens* and C. *rotundus* improved cotton productivity. This corroborates the work of several authors who have found that extracts of H. *suaveolens* and C. *rotundus* reduce pest damage to crops (Fall et al., 2017; Revi et al., 2017; Sane et al., 2018; Vijender *et al*., 2018; and Abakar *et al*., 2020). This protection is thought to be due to secondary metabolites, particularly alkaloids, flavonoids, terpenoids, and phenolic compounds present in these extracts of H. *suaveolens* and C. *rotundus*. Similarly, mycorrhizae introduced into the soil appear to have enhanced the plant's virulence against H. *armigera* (Hamza, 2014; Abakar *et al*., 2020). Furthermore, mycorrhization induces an active state of immunity in the plant, enabling it to be more effective in its responses to pest attacks (Ismail *et al*., 2012; Jung *et al*., 2012; Jochems-Tanguay, 2014). Seed cotton provides vegetable oil for human consumption and protein-rich oilseed cake, which serves as a staple food for livestock. In this study, the plot enriched with mycorrhizae and treated with extracts of *H. *suaveolens** yielded a higher seed cotton yield. The mycorrhizae improved the protection of the cotton bolls against this potential pest. This protection is due to the increased concentration of secondary metabolites in the cotton bolls caused by the mycorrhizae (Abakar *et al*., 2020).

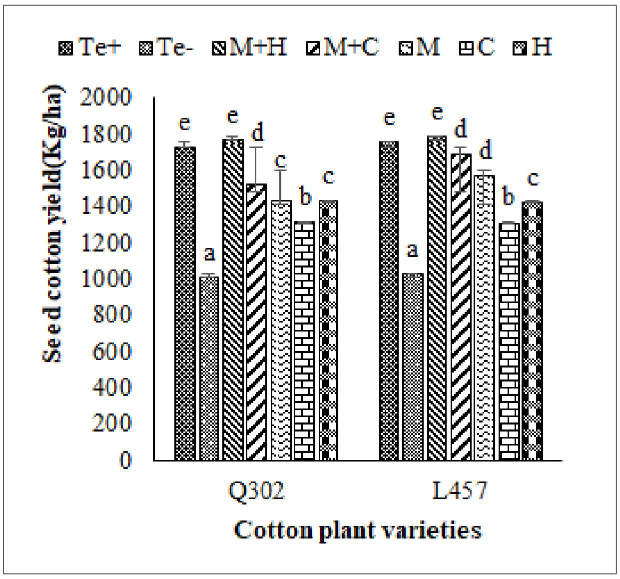

Figure 12: Effects of mycorrhizae and aqueous extracts of *Hyptis suaveolens* and *Cyperus rotundus* on seed cotton yield in the Kodek locality

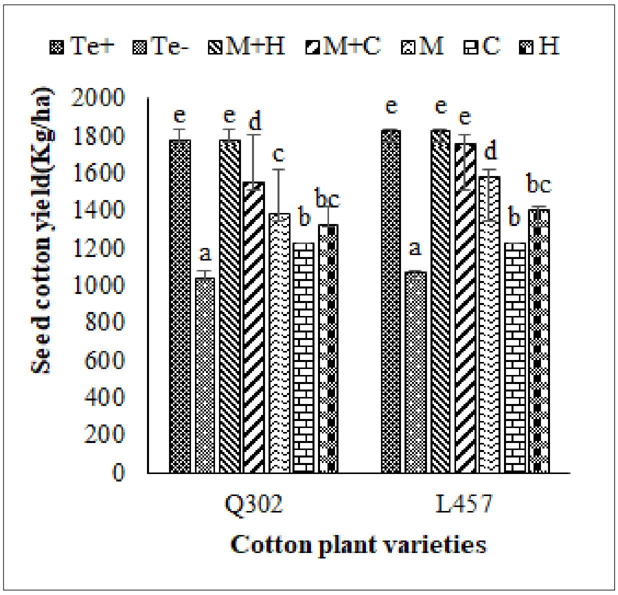

Figure 13: Effects of mycorrhizae and aqueous extracts of *Hyptis suaveolens* and *Cyperus rotundus* on seed cotton yield in the Kodek locality

CONCLUSION

Cotton yields were higher in plots treated with a combination of mycorrhizae and aqueous extracts of Hyptis suaveolens leaves and Cyperus rotundus rhizomes (M+H and M+C) compared to treatments with Te-, H, C, and M. Plots treated with the mycorrhizae and aqueous extract combination improved crop yields while reducing pest impact. The mycorrhizae facilitated the plant's water and mineral uptake, thus improving its productivity.

Similarly, the aqueous extracts used in this study limited the impact of pests on cotton cultivation. M+H and M+C treatments could be a solution for reducing the use of chemical inputs in cotton farming. Inoculating mycorrhizae at sowing and treating with aqueous extracts of Hyptis suaveolens leaves or Cyperus rotundus rhizomes from cotton flowering (45 days after sowing) at regular 7-day intervals until boll maturation could be an alternative to the use of chemical inputs.

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