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RESEARCH ARTICLE

The Comparative Study of Biological and Chemical Foliar Application on Cotton Seedling

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Abstract

The cotton plant is a perennial with an indeterminate growth habit, and reputed to have the most complex growth habit of all major row crops. Cotton, which is grown for using fiber and oil is one of the most important and strategic crops through the world especially in Tamilnadu (India). Exogenous applications of biological and chemical substances have a many potential to promote the plant growth in different way, and the application of different chemicals may reduce stress induced inhibition of plant growth. In these experiments foliar spray of Neem oil was increased the Chlorophyll content (93.24%), humic acid (86.30%), and *Trichodermaviride*(73.50%) compared with others. In plants the Phyllosphere and Rhizosphere both the region was very important. The microbial load in above region was calculated in every treatment in that abundant microbial load was observed in 2A, 3A, 4A, 5A cotton seedling of Phyllosphere area after II treatment. In Rhizosphere region the III and IVth treatments were showed abundant microbial load. And also increased the plant shoot, root length, and Leaf lets number, Leaf size. The activity of these substances on cotton seedlings in every treatment was evaluated by using qualitative and quantitative methods. Spraying the aqueous solution of biological suspension on cotton plants has resulted in enhanced the growth and yield of cotton crops, and chemical spraying decreased the soil fertility. So, this study was carried out to find out the impact of foliar application of biological and chemical substances on cotton seedling was monitored and compared.

Introduction

Cotton is of great commercial importance and is cultivated throughout the world. It is one of the oldest fiber crops. Being an indispensable industrial commodity, it has world demand and thus holds a unique position in international commerce (Hussain and Bano, 2001).Cotton crop is mainly grown for fiber but has many other valuable uses. Cotton seed has 30% starch, 25% semidrying oil and 16-20% protein. Cotton is also an important source of vegetable oil (Ahmad and Makhdoom, 1992).It is also required in edible oil industries. Its cake is used for the food of cattle. A great amount of foreign exchange is also being earned through the export of raw cotton and its products annually. Cotton belongs to genus Gossypium. It has about 41 species but the cultivated cottons are divided into 4 large groups i.e.

1. Gossypium arboretum L. –Desi cotton

2. Gossypiumharbaceum L. – Desi cotton

3. Gossypiumbarbadense L. – Egyptian cotton
4. Gossypiumhirsutum L. – American cotton

About 96% of the total area in the world is cultivated for American cotton and only 4% is cultivated for wild cotton. American cotton is used in industries while wild cotton is used for domestic needs. Cotton plant has a natural mechanism to prevent excessive vegetative growth. In many cases growth regulators are needed to maintain proper plant size and to promote boll set and early maturity (Hunnur, 2007). Manipulation of cotton plant architecture using plant growth regulators can be an agronomic strategy for obtaining high yields (Fernandez et al,1991); (Souza,2007). Plant growth regulators are substances when added in small amounts modify the growth of plant usually regulation, they are considered as new generation of agro-chemicals after fertilizers, pesticides and herbicides (Graham et al,1987).The use of these compounds to reduce plant height in cotton results in earlier maturity and, under some circumstances, increased yield (Hodges et al,1991). Plant growth regulators play a key role in internal control mechanism of plant growth by interacting with key metabolic processes such as nucleic acid and protein synthesis.

Biological control an alternative to the noxious chemical pesticide has attained importance in modern agriculture to curtail the hazards of intensive use of toxic chemicals. This approach is self-sustaining, efficient and ecofriendly with long-term action. Moreover, it is the major component of modern integrated pest management. Several antagonistic microbes have recently been identified as effective biocontrol agents of soil borne plant pathogens (Walsh et al, *2000;* Howell, 2002; Xue, 2003). Among various fungal antagonists, *Trichoderma* spp. have gained wide attention due to their ability to control many fungal pathogens on a variety of crop plants under greenhouse and field conditions (Whipps, 1996) as well as to their growth promotion effects on host plants (Baker, 1988).*Trichoderma* spp. have been known for decades to increase plant growth both shoot and root biomass and crop yield (Harman, 2000) to increase plant nutrient uptake and fertilizer utilization (Yedidia et al, 2001), to grow more rapidly, and to enhance plant green-nests, which might result in higher photosynthetic rate. These same organisms also have been known for a very long time to have the ability to control plant pathogenic fungi (Weindling, 1932).

As foliar spray improved crop proximate and nutritional contents. In bread wheat, the use of foliar urea was reported to increase the protein content of the grain and this may provide the quality benefits of N fertilization and simultaneously reduce risks of nitrate leaching and denitrification. Foliar spays of fertilizer could also be used to manipulate leaf duration, dry matter accumulation and net photosynthetic rate in plants. The spray application technique is a key process influencing the effectiveness of a foliar fertilizer. The application process is complex and involves: the formulation of an active ingredient; atomization of the spray solution; transport of the spray to the target plant surface and droplet impaction; spreading and retention on the leaf surface; residue formation and penetration into the leaf. Application of a foliar treatment implies that the liquid is passed through a spray generating system to produce droplets which are commonly different types of pressure nozzles. The spraying technique strongly influences the performance of a foliar nutrient spray. Chemical control with herbicides has been an important tool for managing weeds in crops and home landscapes for many years. Many of today's herbicides are more effective and selective. These traits make them less harmful to the environment when they are used properly. The glyphosate herbicides affect the amino acid synthesis in a different way. These herbicides are non-selective and control a broad range of annual and perennial grasses, broadleaves and sedges (Baumann et al, 1914).

When plants nutrients are applied to the foliage of the plant, smaller quantities of the fertilizer materials are required than when applying to the soil. The danger of fixation and or leaching is also reduced when nutrients are applied to the foliage of the plant. Nutrients applied to the foliage are generally absorbed more rapidly than when applied to the soil. Foliar application provides a means of quickly correcting plant nutrients deficiencies, when identified on the plant. Soil microbial populations respond to the release of organic materials near the plant root, increasing their members and changing the characteristics of the microbial community. This region called the rhizosphere. A much greater relative stimulation will occur in the nutrient rich rhizosphere of these soils. Rhizosphere microorganisms serve as a labile source of nutrients and also play a critical role in organic matter synthesis and degradation. The agriculture departments insist formers to spray various insecticides and neem oil against pest. The present study has been made to investigate the impact and comparison of foliar application of various insecticides, herbicides, biofertilizers and biocontrol agents on cotton plants.

Materials and Methods

Experimental design

The study was carried out in Muthayammal College of Arts and Science, Rasipuram, Namakkal district in Tamilnadu (India). In this study, impact and comparison of biological and chemical foliar substances on cotton plants was monitored. Find out the Phyllosphere and Rhizosphere microbial population in foliar treated plants. Then monitored the morphological parameters and quantify the biosynthesis of chlorophyll in cotton plants.

Collection of soil and cotton seeds

The 30 kg of Garden soil was collected and 2 kg of soil were filled in 10cm in diameter polythene bags (12nos).The cotton seeds were derived from agro service from Rasipuram.

The collection of biological fertilizers and chemical substances

The *Azospirillum* and *Trichodermaviride* were obtained from Biofertilizer production centre Salem. *Aspergillu sniger* was isolated from soil and cultured in lab. The Herbicide, Insecticide, Biostimulant and neem oil were procured from agro service, Rasipuram.

Seed sowing and rising of seedlings:

Collected cotton seeds were dipped in water containing bowl, floated seeds was removed and settled seeds were selected for seedlings. The equal number of selected seeds was sown in all polythene bags (12nos). Then the holes were makes on the sides of the polythene bags for removing excess of water and for good aeration of seedlings. The seedlings were raised under good condition irrigated with temperature and water daily.

Biological and chemical foliar application

The sowed cotton seeds were very well grown on in the polythene bags. The condition of the cotton plants was morphologically suitable for the treatment of biological and chemical substances. The purpose of this treatment was to protect the plant from Pest, weeds, insects and pathogenic microbes. Then stimulate the plant growth for the production of higher yield. Because the health of the plants is must need for the obtaining the large quantity of production. After 25 days this foliar treatment (sprayed) was applied on respective grown up seedlings.
The following biological and chemical substances used for the above application

I) *Azospirillum* broth culture,
II) *Trichoderma viride*broth culture,
III) *Aspergillus niger* broth culture,
IV) Neem oil (3ml in 100 ml water),
V) Biostimulant low concentration [humic acid powder] (0.5g in 500 ml water),
VI) Biostimulant high concentration (5g in 500 ml water),
VII) Insecticide powder[Malathion 25% wettable powder],
VIII) Insecticide liquid [Imidacloprid 10% SL] (1ml in 100ml water),
IX) Herbicide [Glyphosate] (0.5 ml in 500ml water).

Foliar application methods on cotton plants

The bioinoculum (*Azospirillum*, *Trichoderma viride* and *Aspergillus niger*) were mass cultivated in respective broth culture, then they were sprayed with help of the sprayer. In the same way chemicals (neem oil, herbicide, and insecticides) were also diluted in water and sprayed, care must be taken over the seedlings for sprayed drops not to reach the soil. Foliar treatment must be given to the seedlings in every five days of intervals.

Measurement of growth parameters

Shoot elongation

The seedlings were uprooted carefully without any damage. The shoot length was measured from base of the stem to the last leaf open by using a scale (centimeter).

Root elongation

In seedlings process the growth of root system was developed without any damage. The root length was measured from base of the main stem to end of the root by using scale (centimeter).

Leaf lets number and leaf size

After completion of five foliar treatments, the numbers of leaf lets and its size were measured in the respective plants. Then it was compared with control plant. This monitor process was applied up to the completion of five treatments of regular interval. Finally number and it size measurement was recorded. The leaf let's length and width was measured by scale in cm (length – base of the leaf to tip).

Biochemical estimation

Estimation of chlorophyll

Known amounts of fresh leaf tissues were homogenized in a pre-chilled mortar and pestle with cold 80% acetone. The homogenate was spinned at 4000 rpm for 5 minutes. The supernatant collected and the pellet was re-extracted with some more acetone. The extraction process was repeated until a colorless pellet obtained. All supernatant pooled together and volume measured. The absorbance was read at 645 and 663 in a spectrophotometer. Chlorophyll content was calculated with the following formula.

$$\text{Chl.a (mg/g)} = \frac{(12.7 \times OD663) - (2.69 \times OD645)}{1000 \times W} \times V$$

$$\text{Chl.b (mg/g)} = \frac{(22.9 \times OD645) - (4.68 \times OD663)}{1000 \times W} \times V$$

$$\text{Total chlorophyll (mg/g)} = \frac{(20.2 \times OD643) - (8.02 \times OD663)}{1000 \times W} \times V$$

V= Volume, W= Weight

Isolation of microbial population in rhizosphere by serial dilution

Using sterile spatula, the rhizosphere soil were collected from the near to the plant root of cotton plants (weighed 1 gm aseptically).1 gm of soil sample was taken from respective treated soil and dissolved in separate 100ml of sterile distilled water. This represents 10^{-2} dilution, from the above dilution the soil sample was diluted up to 10^{-7} dilutions. Nutrient agar medium was prepared as per the composition; it was sterilized and dispensed in sterile Petri plates. From the 10^{-2} and 10^{-3} dilution, 0.1 ml of the sample was taken and speeded on nutrient agar medium. Duplicates and controls were maintained. Inoculated plates were incubated at room temperature in dark conditions.

Isolation of microbial population in phyllosphere by leaf imprints method

Each and every treated seedlings leaves was removed from the plants and directly imprint on the Nutrient and Jensen's media. The plates were incubated at room temperature, after the incubation microbial population of phyllosphere was observed.

Results and Discussion

Biochemical and Morphological parameters of cotton seedlings

The foliar applications of biological and chemical fertilizers were sprayed on grown up cotton plants with regular intervals were showed in the (Fig 1). The biostimulant fertilizers were used for soil incorporation before the soil is treated with inorganic fertilizer. The foliar component is given one, two or more applications depending on crops. It produces a substantial increase in yield for various crops and resistance to disease and stress due to temperature, water, wind and pests. Our present findings also similar to that of above findings, biostimulant are increases the microbial population, chlorophyll content, root length and shoot length compare to untreated plants control plants. These yields are highly resistant stress and stress due to temperature, water, wind and pests.

The foliar treated cotton plants were subjected to study their shoot, root elongation and count their leaf lets number, size and its chlorophyll content. The effect of various foliar applications on cotton seedlings showed almost all the chemical foliar treatment enhance the shoot elongation compare to the control but in *Azospirillum* and *Trichoderma viride* foliar treatment not enhanced shoot elongation. (Sairashabeer et al., 2011) discussed about effect of foliar application on nutrients on growth and flowering of *Gerbera jamesonii*L. They said that, the application of macro nutrients solution increases maximum number of leaves per plant (7 no's) compared to control (6.99 no's), (5.77 no's). It also increases the leaf area test 1 (133.18cm), test 2 (119.77cm) compare to the control (92.88cm). The biostimulant fertilizers were used for soil incorporation before the soil is treated with inorganic fertilizer. The foliar component is given one, two or more applications depending on crops. It produces a substantial increase in yield for various crops and resistance to disease and stress due to temperature, water, wind and pests. Our present findings also similar to that of above findings, biostimulant are increases the microbial population, chlorophyll content, root length and shoot length compare to untreated plants control plants. These yields are highly resistant stress and stress due to temperature, water, wind and pests.

In morphological parameters of treated plants shoot length compared with untreated plants shoot length (control- 23.5cm). Neem oil (25cm), biostimulant high concentration (26cm), insecticide powder (24cm), insecticide liquid (24.5cm), these are the treatments enhanced the shoot length. The increased use of glyphosate – resistant crops has raised concerns regarding the potential environmental impact of glyphosate. Foliar – applied chemicals as well as herbicides may impact soil microbial ecology, biostimulants and liquid fertilizers affect soil microorganisms by providing additional nutrients or growth factors that metabolic activity and improve crop growth and productivity. Present investigation also had same findings; herbicide increases the leaf length, shoot length and root length compare to the untreated control plants.

Figure 1 The biological and chemical foliar treated cotton plants

1A- Control Untreated, **2A**- Azospirillum treated, **3A**- *Trichoderma viride*, **4A**- *Aspergillus niger*, **5A**- Neem oil, **6A**- Biostimulant treated (low concentration),**7A**- Biostimulant treated (high concentration), **8A**-Insecticide powder treated, **9A**- Insecticide liquid treated, **10A**- Herbicide treated.

In all types of chemical and biological foliar treatments the neem oil were greatly enhanced the root length compared to the control. Root length of treated cotton seedlings compared with untreated cotton seedlings (25.2cm), neem oil (35cm), biostimulant high concentration (28.2cm), insecticide powder (28 cm), insecticide liquid (29cm) and herbicide (26cm). These are the treatments enhances the root length.(Tadeusz et al., 2002) reported that the unformulated conidia of the fungus *Beauveria bassiana* were applied to potato plots with and without the hemipteran predator perillus bioculantus in an attempt to reduce field populations of *Colorodo potato* beetle, *Leptinotarsa decemlineata*, and to compare their efficacy to chemical insecticide control. Our present investigation also similar to that of Tadeusz findings, because the foliar spraying of *A. niger* on cotton leaves increased the number of beneficial microbial population there is no defoliation and necrosis and other infection compare to neem oil treated plant.

There is no difference in leaf lets percentage, but insecticide supplication enhance the leaf lets and continues application of neem oil and herbicide, induce the defoliation. Initially first application of herbicide damaged to the foliage. In neem oil application after second treatment if shows better leaf size but continuous application induce defoliation. Untreated control plants shows leaf width of (6cm) and leaf length of (6.5cm). It is compared with treated plants. Leaf width of biostimulant high concentration (6.3 cm), insecticide powder (6.4 cm) and insecticide liquid (6.5 cm) shows increased size. Leaf length of *Azospirillum* (6.5cm), biostimulant high concentration (7.5cm), insecticide liquid (7.5cm) and herbicide (7cm) showed increased size compare to control plants. These results were compared with previous work of (EI-Kalla et al., 2006) conducted the two fields experiments during 2003 and 2004 seasons, they used *Azospirillum* and biofertlizers in both seasons, times of nutrients foliar application had a significant effect on flag leaf area, plant height, number of tillers and number of panicles, maximum yield and yield components were produced foliar spraying of green house after 45 days from transplanting in both seasons. My present study also had same findings foliar treated plants like insecticide powder (5 no's) and insecticide liquid (5 no's) increased the number of leaf let's compare to untreated plants (control) (4 no's).

Various treatments on cotton seedlings showed various results, treatment on leaflets, to determine whether the chlorophyll content of the cotton plants was increased or decreased. The chlorophyll percentages are found to be almost lower in all types of treatment compared to the control. So it indicates that foliar applications reduce the chlorophyll synthesis. Biochemical estimation of chlorophyll content of the foliar treated plants compared with untreated control plants. According to formula it was calculated, in chlorophyll content A compare to control (0.0011 mg/g) all treatments decreases the content of chlorophyll, among that *Azospirillum* highly reduce the chlorophyll content (0.0004 mg/g). In chlorophyll content B, neem oil shows increased content of chlorophyll (0.00053) compare to control (0.00052 mg/g) and other treatments. (Fraser and Percival, 2003) said that four commercially available biostimulants sold improvements in tree vitality were assessed by measurement of a chlorophyll a performance index based on leaf chlorophyll fluorescence emissions. Only the biostimulant increased chlorophyll fluorescence values of all test species. Our present findings also had similar findings of the above, because compare to other foliar treatments biostimulants high concentration and neem oil has high chlorophyll content. These different parameters and their results have been represented in (Table 1).

Table 1 Biochemical and Morphological parameters of cotton seedlings

S.No	Foliar treatment	Chlorophyll content (mg)			Shoot length %	Root length %	Leaf lets %	Leaf size	
		Chl.A (mg/g) %	Chl.B (mg/g) %	Total chl. (mg/g) %				Width (cm)%	Length (cm) %
1	1A-Control	100	100	100	100	100	100	100	100
2	2A-Azospirillum	36.36	40.38	38.13	97.87	97.22	100	91.66	107.69
3	*3A-Trichoderma viride*	72.72	73.07	75.00	80.85	71.42	100	78.33	84.61
4	*4A-Aspergillus niger*	54.54	51.92	56.25	97.87	83.33	100	78.33	84.61
5	5A-Neem oil	86.36	101.9	93.75	106.38	138.88	100	-	-
6	6A-Biostimulant (low Concentration	79.09	98.07	87.50	97.87	86.00	100	91.66	92.31
7	7A-Biostimulant (high concentration)	73.63	59.61	68.75	110.63	111.9	100	105.00	115.38
8	8A-Insecticide powder	78.18	63.46	62.50	102.12	111.11	125	106.66	100.00
9	9A-Insecticide liquid	83.63	78.84	81.25	104.25	115.08	125	108.33	115.38
10	10A-Herbicide	72.72	55.76	68.75	102.12	103.17	Then-rebuds	100	107.69

Microbial analysis in rhizosphere and phyllosphere region

In rhizosphere, microbial populations were increased by *Azospirillum, Trichoderma viride, Aspergillus niger,* when compared to insecticide liquid and herbicide treated plants. (Mathivanan and Srinivasan 1997) reported that control of cotton root borne disease was investigated using carbendizim and *T.viride* in the field. Seed treatment with *T.viride* reduced plant mortality by 56% *T.viride* used first as a seed dressing. Followed by soil application, it reduced mortality by 86% integrated use of *T.viride* and carbendizim reduced plant mortality by up to 72%. Foliar application of *T.viride* increases the microbial population in phyllosphere and rhizosphere, enhances the shoot, root and leaf size it was more or less similar to that of above findings. In phyllosphere, all the biological foliar treatments enhance the microbial population compare to control and chemical foliar treatments was given below as in (Fig 2 ,Fig 3).

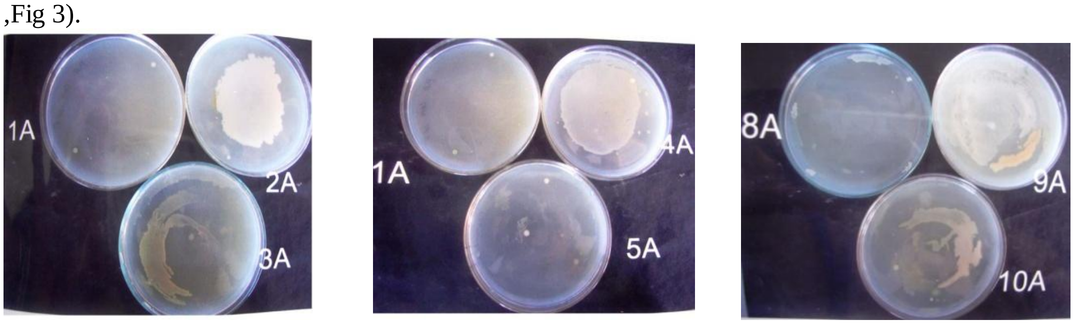

Figure 2 Microbial population in Rhizosphere region by spread plate method (After I, III, IV treatment) from (10^{-5}) dilution

1A- Control Untreated, **2A**- Azospirillum treated, **3A**- *Trichoderma viride,* **4A**- *Aspergillus niger,* **5A**- Neem oil, **8A**-Insecticide powder treated, **9A**- Insecticide liquid treated, **10A**- Herbicide treated.

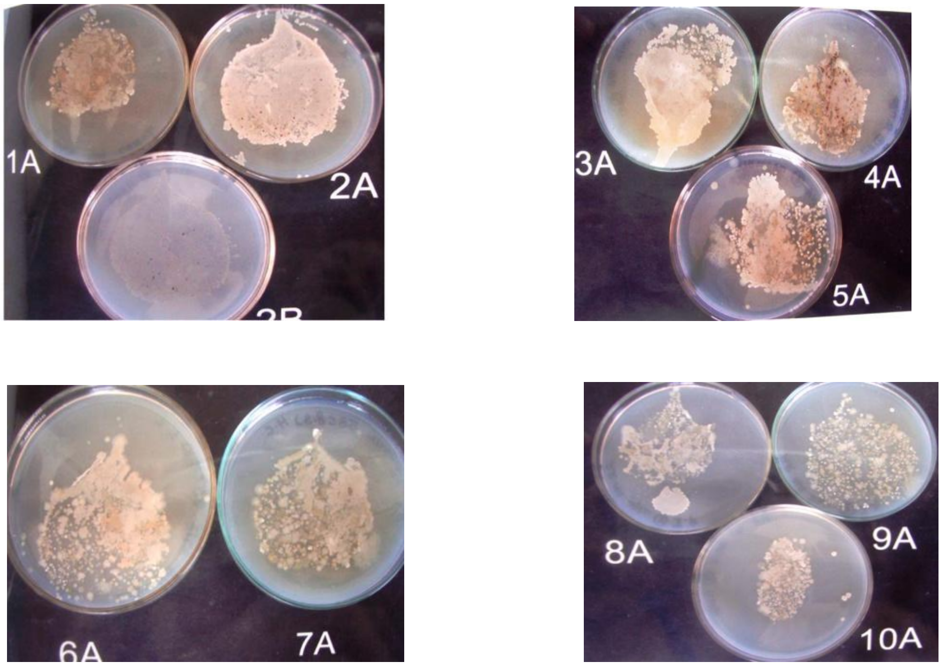

Figure 3 Microbial population in phyllosphere region of cotton plants (After 2^{nd} treatment)
1A- Control Untreated, **2A**- Azospirillum treated, **2B**-*Azospirillum* Jensen's medium, **3A**- *Trichodermaviride*, **4A**- *Aspergillusniger*, **5A**- Neem oil, **6A**- Biostimulant treated (low concentration),**7A**- Biostimulant treated (high concentration), **8A**-Insecticide powder treated, **9A**- Insecticide liquid treated, **10A**- Herbicide treated.

Impact of foliar application in Phyllosphere and rhizosphere region

First foliar treatment

In the first treatments, the rhizosphere microbial populations were enhanced by *Azospirillum*, *T.viride*and insecticide liquid only. Remaining other treatments reduced the population.

Second foliar treatment

The biological treatments *Azospirillum*, *T.viride*, *A. niger*, and neem oil sprayed leaves have heavilypopulated.The remaining all chemicals sprays reduce the population.

Third foliar treatment

The third treatment were enhances the rhizosphere population (10^{-5}dilution) *Azospirillum*, *Aspergillusniger*, biostimulant (both concentration) and insecticide liquid. The remaining other foliar treatment reduce the rhizosphere population such as *T.viride,* neem oil, insecticide powder and herbicide.

Fourth foliar treatment

The effect of foliar treatment on rhizosphere after the fourth treatment following applications were enhanced the microbial population (10^{-4}dilution). *Azospirillum*, *T. viride*, *A. niger*, biostimulant (both concentration) and insecticide powder. The remaining other foliar treatment reduce the rhizosphere population in continuous application such as neem oil, insecticide liquid and herbicide. These all treatments results were showed in (Table 3).

S.No	Foliar treatment	Microbial population in rhizosphere			Phyllosphere after II treatment
		After I treatment	After III treatment	After IV treatment	
1	1A	+++	+++	+++	+++
2	2A	+++++	+++++	+++++	+++++
3	3A	+++++	++	+++++	+++++
4	4A	++	+++++	+++++	+++++
5	5A	++	++	++	+++++
6	6A	++	+++++	++++	+++
7	7A	++	+++++	+++++	+++
8	8A	++	++	+++++	+++
9	9A	++	+++++	++	+++
10	10A	++	+	+	+++

Table 3 Microbial population of rhizosphere and phyllosphere in cotton seedlings

+++++ - Abundant microbial load
++++ - High amount of microbial load
+++ - Moderate microbial load
++ - Reduced microbial load
+ - Poor microbial load

Conclusion

The manipulation of cotton plants using biological and chemical substances can be an agronomic strategy for obtaining high yields. Our results indicated that the different concentrations of chemical foliar application decreased the stem length, leaf number and leaf area in comparison with control plant and biofertilizer treated plants. The results from the foliar application of liquid biologically active culture showed that it exerts a favorable influence on the cotton growth and productivity. No negative effects were seen as a result of applying the biological foliar fertilizer on the cotton plants.

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