Discussion

5. GENERAL DISCUSSION AND CONCLUSION

Root rot and damping off diseases are considered to be one of the most dangerous problems facing cucumber growers due to the great damage of infecting seedlings

The use of fungicides are not the most desirable means of disease control as they are expensive, cause environmental pollution and may induce pathogen resistance (Larson, 1987). Seeds are ideal for delivery of the biological control agents that control seed rots and damping-off diseases because the microorganisms placed directly on the infected seed coat, and a microorganism that colonizes roots is ideal for use as biocontrol agent against soil born disease, (Weller, 1988)..

Application of biofertilizer with cucumber in order to control such diseases and improve the growth and production was conducted in this work. The experiment were carried out in the greenhouse at DRC. Soil used was sandy textured Table (1) collected from 10 th of Ramadan, Cairo, Egypt .

The soil amended with 1% sheep manure to modify its structure and waler holding capacity and supplemented with the recommended doses of nitrogen, phosphorus and potassium Organic matter serves as a source of energy for the development of microorganisms and supplying them with certain essential nutrients required for their growth activity (Boyar, 1963 and Bear, 1965).

In the present study, the causal fungus was isolated from roots of diseased cucumber plants grown in Noubaria (Behera governorate) was identified as *Rhizoctonia solani* (Kuhn) Nitrogen fixers were isolated from soil and rhizosphere of different plants namely cucumber, foeniculum, wheat, corn, barley, cuminum and ocimum. The most active ones in N_2-fixation, root colonization and antagonizing *R. solani* were identified as *Azotobacter chroococcum* (one strain) and *Azospirillum lipoferum* (one strain).

Actinomycetes were isolated from soil and rhizosphere of different plants namely foeniculum, wheat, cuminum, corn, ocimum and cucumber. The most active one in root colonization and antagonizing *R. solani* was identified as *Streptomyces* lydicus (one strain).

The obtained results proved that *Rhizoctonia solani* caused damping-off and root-rot diseases to cucumber plants. *R. solani* decreased the number of survival plants in the greenhouse experiment. Whereas, asymbiotic N_2-fixers *Azotobacter chroococcum* and *Azospirillum lipoferum* and *Streptomyces lydicus* strains significantly reduced the harmful effects of *R. solani* on cucumber plants by reducing the pre and post emergence damping-off, root-rot diseases and DSI of infected plants.

The obtained results obviously revealed that *Streptomyces lydicus* gave highly significant reduction in disease severity and increased plant survival more than those of treated with *Azotobacter chroococum* and/or *Azospirillum lipoferum* or as a mixture. Chet et al. (1990) stated that the biocontrol agents may act against pathogens by one or more of the following mechanisms: competition, antibiotisim, parasitism or predatism as well as induction of resistance in the plants.

These findings were previously interpreted by **Schmiedeknecht (1993); Song** ***et al (1998)*** **and Chamberlain and Crawford (1999)** reported that *Streptomyces* sp. produced antibiotics which controlled root-rot diseases caused by *R. solani.* **Kundu and Nandi (1984)** stated that the reduction of damping off caused by *R. solani* with inoculation with *Streptomyces arenae* and *Streptomyces chibaensis* is due to increase population of the *Streptomyces spp.* leading to lower population of *R. solani.*

With respect to root colonization **Weller (1983), Loper** ***et al.*** **(1984), (1985) and Lifshitz** ***et al.*** **(1986)** found that an effective biological disease control organism should rapidly colonize the root zone. **Weller (1988)** found that a microorganism that colonizes roots is ideal for use as biocontrol agents against soil born disease. **Nemec** ***et at*** **(1996)** found that the biocontrol agents are highly root cololnizers to their hosts.

With respect to the microbial content, the results revealed that each of the total microbial counts, counts of *Azotobacter, Azospirillum* and actinomycetes increased during plant growth reach its maximum at flowering stage then decreased towards harvesting. This may be due to the shortage of biological nitrogen during the maturity stage of plant growth **(Nelson,** 1983).

Also, *Azospirillum* gave the highest microbial counts comparing to those of *Azotobacter* and actinomycetes due to its highest colonization ability to cucumber root .

The inoculation process with *Azotobacter chroococcum* and/or *Azospirillum lipoferum* or *Streptomyces lydicus* or as mixture di or tri caused considerable increases in all the counts of microorganisms in unifested one more than infested soil with *R. solani.* However the microbial growth was enhanced by inoculation with a mixture of *Azotobacter chrococcum + Azospirillum lipoferum + Streptomyces lydicus.*

Several investigators used biofertilizers to improve soil properties to the most convenient ones for the growth of different plants and their rhizospheric microorganisms, **Lynch and White (1977), Kloepper and Schroth (1981), Hassouna** *et at* **(1990) and Kloepper (1992).** They also indicated that rhizobacteria can produce plant growth promoting substances of a biological control effect against soil born disease.

Plant growth as plant height, root length, fresh and dry weight of plant, number of flowers and fruits, weight of fruits, chlorophyll content and nitrogen and protein —percent as affected by inoculation either with *Streptomyces lydicus* or N_2 fixing *(Azotobacter chroococum* or *Azospirillum lipoferum)* single or as mixture di or tri were also determined in infested soil with *R. solani* and uninfested one. All these parameters were evaluated at different stages of cucumber plant growth, i.e. vegetating, flowering, fruiting and harvesting stages.

The obtained results apparently showed that "biofertilizers biocontrol agents" protected plants from the pathogen infection to different e xtents. These results are in line with the finding of **Tahvonen (1988)** who found that seed treatment with powdery preparation of *Streptomyces* not only control root diseases, but also increase yield of cucumber and carnation by 10-30%. The yield increase have been often occurred towards the end of the growing period. **Jizba and Prokinova (1998)** reported that the macrotetrolide compounds isolated from *Streptomyces globisborus* increase the growth of cucumber seedlings.

Gras and Fernendez (1993) found that inoculation with *Azospirillum lipoferum* increase yields of cucumber by 57-114%.

Hassouna *et al* **(1998)** found that inoculation of cucumber with *Azotobacter chroococcum, Azospirillum brosilense* and *Klebsiella pneumonia* increases the cucumber photosynthetic area by 22%, N2-content by 89% and shoot and root dry weight by 30 and 80%, respectively.

Fouad (1981), Girgis (1985), Nieto and Frankenberger (1989) and Arshad and Frankenberger (1991) found that azotobacters and azospirilla produced plant growth substances namely indole acetic acid (IAA), indole lactic acid (ILA), gibberellin, cytokinine, L-treptophan (LTRP), auxins and a tenine isopentyl alcohol like substances.

The positive influence of associative N_2-fixers *(Azotobacter* and *Azospirillum)* on plant development can be atributed not only to the N_2-fixation process, but also to the production of growth promoting substances and the production of antifungal antibiotics **Vlassak and Reynders (1980), El Haddad** *et aL* **(1986, a) and Ishac** *et al.* **(1989).**

It can be concluded that application of a mixed culture of *Azotobacter chroococcum + Azospirillum lipoferum + Streptomyces lydicus* appreciably reduces the infection with *R. solani* and in the same time increases the growth and yield of cucumber plants to considerable extents by providing the growing plants with growth promoting substances as well as their antogonistic effect against fungal disease which cause great yield losses.

The obtained results can be summarized as following :

1. A number of 117 N_2-fixing diazotrophs and actinomycetes have been isolated from different localities of Egyptian soils cultivated with different plants. These isolates were 25 N2-fixing diazotrophs and 11 actinomycetes.
2. Pathogenic fungus was isolated from rotted cucumber roots collected from Noubaria city and identified as *Rhizoctonia solani.*
3. The nitrogen fixation ability of *Azotobacter* and *Azospirillum* were tested. Also, antagonistic activity of *Azotobacter, Azospirillum* and actinomycetes were tested and the most active isolates of *them* (2 isolates of each) were chosen.
4. *Azotobacter* isolate Rf (isolated from 10 th of Ramadan soil cultivated with Foeniculum and *Azospirillum* isolate lc isolated from El Khatatba soil cultivated with cuminum proved to be the most active isolates for nitrogen fixation (280 ppm and 133 ppm), respectively. Actinomycetes isolate, Non isolated from Noubaria soil cultivated with cucumber, *Azotobacter* Rf and *Azospirillum lc* were the most active isolates in antagonizing the pathogenic fungus. (The inhibition zone diameter were 22, 15 and 17 cm respectively.
5. For root colonization ability the three strains Rf, Ncu, were the most active ones and ranged descendingly as *Azospirillum* sp. lc (63.5%), *Azotobacter sp.* Rf (62%) and *Streptomyces* sp. Ncu1 (41.4%).

6. These isolates were identified as Rf *(Azotobacter chroococcum)*, *(Azospirillum lipoferum)* and $N_{ai}l$ *(Streptomyces lydicus)*

7. Pathogenic fungus isolated from rotted cucumber roots was identified as *Rhizotonia solani.*

8. Inoculation with N2-fixers and *Streptomyces lydicus* reduce the disease severity index (DSI) of infected cucumber plants from 75% to 50%.

9. Inoculation with strains Rf Kc, Neu, , individual or in a mixture di or tri stimulated the growth of total microbes in infested and uninfested sandy soil cultivated with cucumber plants. The highest total microbial count was noticed in inoculated treatment with a mixture in unifested and infested soil being 276, 240 x 10^4 cfu/g dry soil comparing with control treatment being 128, 112 x 10^4 cfu/g dry soil at fruiting growth stage, respectively.

10. Inoculation with a mixture of N2 fixers diazotrophs (R_f + Kc) and *Streptomyces lydicus* $N_a$il increased actinomyces counts to considerable extents. The highest counts of actinomyces (99 and 87 x 10^3 cfu/g dry soil) were recorded in infested and uninfested soil at fruiting stage of cucumber plant growth, respectively.

11. Inoculation with strains Rf, $N_{ai}l$ as a mixture increased azotobacters, density. The highest densities of azotobacters (1.1, 0.84 x 10^4 cells /g dry soil) were recorded in infested and uninfested soil applied with a mixture of IC, + $N_a$il + Rf at fruiting stage of plant growth, respectively.

12. Inoculation with Rf Kc, Neill strains reduced the effect of *R. solani* on azospirilla densities as the densities in infested soil less than uninfested one . The densities of azospirilla in inocualted treatments wee more than control. The highest densities (1.3, 1.7 x 10^4 cells /g dry soil) were recorded in soil applied with a mixture of (Rf + Ke +New) strains in infested and uninfested soil at fruiting stage, respectively.

13. Inoculated treatments increased cucumber plants height from 113.6 cm in control uninfested soil to 160.1 cm using a mixture of biofertilizer. However cucumber plants cultivated in infested soil were shorter than, those cultivated in uninfested ones being 87.8 cm in infested control soil and 124.7 cm using a mixture inoculation.

14. Inoculated treatments gave the highest root length being 38.1 and 43.7 cm in the presence of a mixture of Rf, Kc, Neu , strains for infested and uninfested soil at harvesting stage of plant growth, respectively.

15. Inoculation with a mixture strains under study gave remarkable increases in the stem fresh weights being 37.2 and 45.5 gm and 3.5 and 5.2 for stem dry weights in infested and uninfested soil at harvesting stage of cucumber plant growth, respectively.

16. Inoculation with tri strains Rf + + Ne., decreased the harmful effect of pathogenic fungus *R. solani* in fresh or dry weights of cucumber roots. The highest fresh weight were (4.85 and 5.9 gm) for infested and uninfested soil at harvesting growth age of

cucumber plants, respectively. The corresponding figures for dry weights of roots were 0.91 and 1.2 gm.

17. The highest chlorophyll content was at flowering stage in uninfested soil 47.9% using a mixture of Rf, lc, N_ed but the lowest one was 44% with Streptomyces inoculation comparing with control 35.6%, while infested control soil recorded only 33.3 % .
18. Inoculation with a mixture strains increased cucumber flowers and fruits number in uninfested soil comparing with infested one (54.6, 39.3) flowers/plant and (22.7, 16) fruits/plant.
19. Cucumber plants cultivated in inoculated treatments had fresh weight for fruits more than uninoculated. Inoculation treatments decreased the reduction in fruits fresh weight caused by infection with *R. solani* for plants. The maximum weight was 297.3 gm/plant using a mixture strains in uninfested soil. For infested one inoculation with Rf, K_e and N_ed increased the length to 201.6 gm/plant comparing with infested control 83.9 gm/plant.
20. Cucumber plants cultivated in infested soil had lower fruit N-content and protein than those cultivated in uninfested ones.
21. The highest total N and protein % of cucumber fruits cultivated in uninfested soil treated with mixture strains were 1.52; 9.5% but for infested soil were1.4; 8.75 %, respectively.