

Comparison of natural soil sterilization methods and their effects on soil inhabitant fungi

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Abstract: The most frequently soil-borne fungal pathogens on plants that make high economical damages in various agricultural locations from Libya and the world. The temperature and method for killing soil organisms in soil are so important factors that affect the results. The aim of this study was to compare and evaluate the effectiveness of some non-chemical methods of soil sterilization: Solar heating, Hot water and Flooding for sterilization of lentil nursery seedbed soil. Treatments resulted difference of types and numbers of fungi before and after treatment. Protagules of *Fusarium oxysporum*, *Fusarium solani*, and *Pythium* sp were greatly reduced or completely eliminated in soil treatment. Soil irrigation + covered with transparent and Hot water were more effective than the other treatments to control soil borne fungi. Seedlings survival were increased to 90 and 100% after treatment with Soil irrigation + covered with transparent and Hot water respectively compared to 75% for the plants were sown in non-treated pots. These methods was simple, effective, non negative side and applicable in diverse farming areas at warm season.

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1. Introduction

Soil contains a vast array of microorganisms such as bacteria, viruses, fungi, actinomycetes, protozoa and algae (Alexander, 1977; Olowonibi, 2003). Soil organism participates in the genesis of the habitat, which they live. They, together with the total biota and especially the higher vegetation, constitute one of the five interactive factors in soil formation; the other four are climate, topography, parent material, and time (Beare, 1997). Also, Soil contains weed seeds, which, when germinated, compete with garden plants for space, sunlight, and nutrients. Such competition weakens plants, making them more susceptible to attack by insects and disease-causing organisms such as nematodes, bacteria, and fungi. A severe infection may even kill the plant. Methods of Controlling Soil Organisms One of the more effective ways to control these diseases and pests is to treat the soil by either physical or chemical means. In each case, complete or partial treatment may be given. soilborne pests can be controlled in vegetable and fruit crops by preplant application of pesticides, including the fumigants methyl bromide, chloropicrin, and metam sodium. The use of these materials, however, is often undesirable due to their toxicity to animals and people, their residual toxicity in plants and soils, the complexity of soil treatment, and their high cost. Furthermore, restrictions on the use of soil-applied pesticides seem imminent as existing environmental legislation is implemented. As a result, there has been an increased emphasis on reduced-pesticide or nonpesticidal control methods.

Soil solarization is a nonpesticidal method of controlling soil borne pests by placing plastic sheets

on moist soil during periods of high ambient temperature (Elmore et al., 1997). The plastic sheets allow the sun's radiant energy to be trapped in the soil, heating the upper levels. Solarization during the hot summer months can increase soil temperature to levels that kill many disease-causing organisms (pathogens), nematodes, and weed seed (Pullman et al., 1981; Pinkas et al., 1984; Annesi and Motta, 1994; Stapleton et al., 2000 a,b; Ashrafi et al., 2010; Handiseni et al., 2010; Saremi et al., 2010; 2011). Soil solarization is obtained by covering soil with plastic films, a useful practice able to reduce soil pathogen populations. Actually, light plastic films (LPFs) are nowadays widely used especially in open and greenhouse vegetable crop cultivations in some countries as they are able to raise soil temperature more than 20°C above air temperature (Gonzalez et al., 1993; Kumar et al., 2002; Elabiedy and Ibrahim, 2012). It leaves no toxic residues and can be easily used on a small or large scale. Soil solarization also improves soil structure and increases the availability of nitrogen (N) and other essential plant nutrients.

Soil can be heated with steam, hot water, or dry heat in an oven or similar device, or by passing a high-amperage electric current through the soil. Steam or hot water is most effective, as it hydrates the weed seeds, pathogens, and insects, making them more susceptible to the heat. (Uematsu, et al., 2003). The hot water treatment has recently received special attention in Japan as the most promising Methyl bromide alternative (Kita, et al., 2003). Application of hot water (95 to 100°C) onto soil surface raise the soil temperature up to the lethal level of the plant pathogens as well as pests and weed seeds (Kita, et

al., 2003; Fujinaga, *et al.*, 2005 and Ogawara, *et al.*, 2006). Mahdy *et al.* (2011) reported that complete reduction in total count of *Rhizoctonia solani*, *Fusarium solani*, *Sclerotium rolfsii* and *Pythium ultimum* fungi and root rot disease of cucumber was obtained with boiling water as soil treatments at 0.4 & 0.5 l / kg soil and hot water at 90.0 & 100 °C at rate 0.5 l / kg soil. It could be suggested that hot water as soil treatments might be safely used commercially as a new approach for controlling root rot disease of cucumber plants under greenhouse conditions flooded with water for 2 weeks then 2 weeks dryin. Soil anaerobic conditions in flooded plots were monitored with oxidation-reduction. The oxidation-reduction potential increased to aerobic soil conditions within 4 days after the flooded plots. The reduction of O₂ in soil was attributed severance exchange the gases with the air as well as the organisms consumed all O₂ in soil. Results of flooding were reduction of nematode, and other harmful organisms such as bacteria, fungi and insects (Nelson *et al.*, 2000).

This study has devoted to evaluation the outputs of soil sterilization with different methods on soil inhabitant fungi and Management by selection method which is simple, effective, has no negative side effect, an economic technique and can be used in nearly different areas.

2. Material and Methods

Background of the research location:

A field experiment has been conducted at the Farm of Horticulture Department , Faculty of Agriculture, Omar AL-Mokhtar University , AL-Beida, AL-Gabal AL-Akhdar region. The area lies between lat 30° 45' and 30°16' S, and long 21°42' and 21°37.9' E with an altitude ranging from 612 ± 8.4 meters above mean sea level. soil of the site is a typical clay soil, composed of 11.8% sand, 35.2% silt and 50.1% clay.

Soil samples:

In the summer of 2013, triplicate soil samples were randomly collected from depth 20cm, by using a sterile auger. soil samples were taken from three distinct points with five meters between two points. and mixed to give a composite sample in order to determine the fungal population density and evaluated solarization methods for disinfecting soil from soil borne fungi. Each soil sample was kept in clean plastic bag at 4-6°C until plated.

Isolation of fungi:

Fungal soil populations were estimated after soil sterilisation treatments and a non-sterilized sample was used as a control. In the lab, the soil were cleaned and removed the gravel, Plant debris, remnants of roots and seeds of weeds then crushed and sifted. By

using a sterile Spatula 0.5gm were transferred aseptically to 9 mm sterile Petri dish. Ten ml of PDA medium (45°C) were added, hand swirled and left to solidify, being that the dishes were incubated for one week at 25-27°C in the dark. The number of fungi colonies present was assessed in the third and seven days of incubation. As soon as the fungal growth appeared, colonies transferred PDA to obtain pure cultures and maintain them. Colony diameters were measured at 7 days in the mentioned media. Stereomicroscope and light microscopes were used to determine the colonial features and the morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on material mounted in lactophenol.

Pure culture of the isolated fungi were identified according the following descriptive manuals, Raper and Fennell (1965); CMI(1966); Rifai (1969); Nelson *et al.* (1983); Pit (1985); Malone and Muskett (1997); Barnett and Hunter (1999) and Mathur and Kongsdal (2003).

Preparation of soil for sterilization:

After studying fungal diversity in soil samples. The soil divided into eight groups (Plate 1) in seedbeds (50 cm × 30 cm × 10cm). soil was sterilized by three methods:

1- Solar heating:

This ensured that the plastic is held in place and will heat from escaping and allowing wind to set beneath. To increase the transmission of heat through the soil and make other resting structures more sensitive to high temperature, soil smoothly maintained on the surface and irrigated prior to solarization to the saturation level to facilitate better solar heat movement. Black (B), Green (G) and Transparent (T) plastic polythene of 100 m thickness was used to cover the appropriate experimental seedbeds. The other three groups were covered without watering. All seedbeds were leaved in the field during the period from 15/6/2013 to 31/9/2013. After 107 days of solarization the covers polythene sheet was removed.

2- Hot water:

One liter of boiling water (100 °C)/ kg soil applied as soil treatment were tested to study their effect on total count of soil borne fungi. After 24h the water excess was removed and soil was dried for 7 days.

3- Flooding:

One liter of water was poured on soil (1L/1kg) then leaved to two week in extra water. After 14 days The water excess was removed and soil was dried for 14 days.

Soil samples were taken immediately after sterilisation of seedbeds. Soil samples of approximately 200 g were collected from three

randomly selected points in each seedbed. The soils for each point and from the same seedbed were combined and stored in a khaki paper bag to constitute one sample.

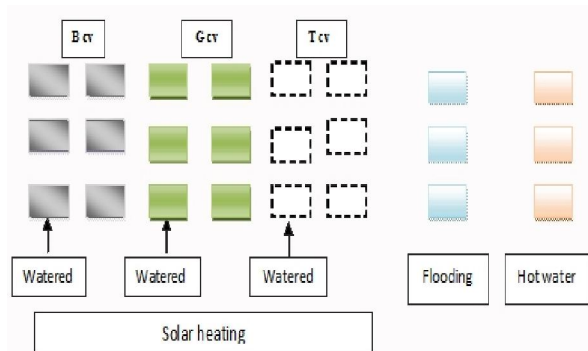


Plate 1. Design of experiments.

Seed sowing:

Lentil (*Lens culinaris*) seeds were sown on pots after filling with soil from seedbeds. The pots were sown by hand drilling 10 seeds/pots. Watering was done three times daily at 08:00 H, 12:00 H and 17:00 H with a watering can fitted with a fine sprayer until seedling emergence. After emergence the pots were watered twice daily at 08:00 H and 17:00 H.

Disease incidence and seedling survival:

Disease incidence was assessed in the nursery seedbeds beginning 4 weeks after sowing. Seedling mortality was assessed by counting the number of seed decay and seedlings dying after germination and expressing it as a percentage of seedlings that had germinated two weeks after sowing. Data on the Disease incidence was subjected to angular transformations before analysis. All data were statistical by use Co stat program.

3. Results:

Results in Table (1) shows the list of fungi in soil samples. A total thirteen species belonging to eleven genera were isolated from pre- treated and post-treated soil. From obtained results it could be noticed that, some of genera were recorded pre- treatment such as *Alternaria alternate*, *Aspergillus niger*, *Mucor* sp, *Phoma* sp, *Rhizopus nigricans*, *Trichoderma viride* and *Ulocadium botrytis*, and other genera such as *Cheatomium fimeti*, *Penicillium* sp. and *P. digitatum* were recorded post-treatment, Meanwhile *Fusarium solani* and *F. oxysporum* were found pre- and post-treatment. Micromorphological structures of the predominant fungi are illustrated in plates. 2–13.

The most dominant one among the isolated fungi was found to be *Fusarium* (56.3%), which was followed by *Trichoderma* (12.7%), while *Alternaria*

and *Aspergillus* reached to 7.04% (Plate 14). The least frequency (1.41%) was recorded by *Cheatomium*, *Phoma*, *Pythium* and *Rhizopus*.

Table 1: Presence of fungi in soil pre- and post treatment.

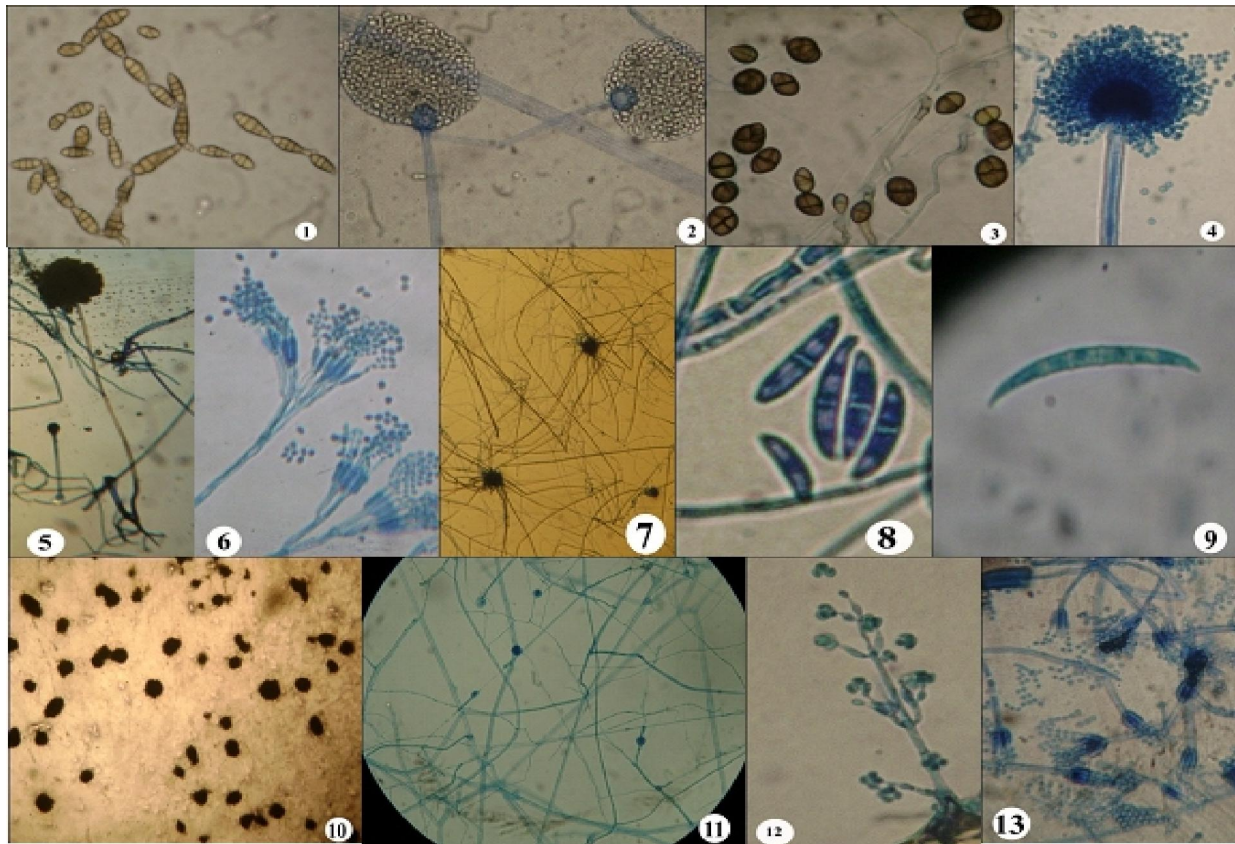
Fungi	Pre-treatment	Post-treatment
<i>Alternaria alternate</i>	+	-
<i>Aspergillus niger</i>	+	+
<i>Cheatomium fimeti</i>	-	+
<i>Fusarium oxysporum</i>	+	+
<i>Fusarium solani</i>	+	+
<i>Mucor</i> sp	+	-
<i>Phoma</i> sp	+	-
<i>Penicillium digitatum</i>	-	+
<i>Penucillum</i> sp	-	+
<i>Pythium</i> sp	+	-
<i>Rhizopus nigricans</i>	+	-
<i>Trichoderma viride</i>	+	+
<i>Ulocadium botrytis</i>	+	-
+: found, -: not found		

According to the data obtained from 9 different treatments (Table 2), it is clear that change the percentage was observed in the density of fungi depending on the treatments. Fungal density in the control (non-treatment) recorded the highest level, while the decline of fungal density was obtained in other treatments. The predominant fungal species in the post-treatment soil were *Fusarium oxysporum* (32.4%) and *Fusarium solani* (10%). The genus of *Cheatomium* and *Penicillium* was observed after treatment only.

The irrigation treatments were most effective in reduction inoculums of fungi than non irrigation. From the data (Table 2), the results indicated that the treatment with irrigation + transparent cover and the treatment with flooding were most effective in suppression of all fungi.

Results in Table (3) indicate that all treatments of soil serialization significantly reduced the seed decay incidence and enhanced seedlings survival compared with control. The most effective treatments are Solarization + transparent cover + Irrigation and hot water which reduced the seed decay disease (0%). It recorded that 90 and 100 % for seedlings survival respectively. Moderate effect (80%) was obtained with Solarization + Green cover + Irrigation which reduced the disease incidence. While, treatment without irrigation showed less effect.

Concerning with the disease severity Data in Table (4) show that all sterilization treatments decrease the disease severity from 0.39 in control to 0.0 in hot water treatment.



Plates 2- 13: Micromorphological structure of *A. alternata* (1), *Mucor* sp (2), *U. botrytis* (3), *A. niger* (4), *R. nigricans* (5), *P. digitatum* (6), *C. fimeiti* (7), *F. solani* (8), *F. oxysporum* (9), *Phoma* sp (10), *Pythium* sp (11), *T. viride* (12) and *Penicillium* sp (13).

4. Discussion:

An experiment has been conducted at Faculty of agricultural Omar AL-Mokhtar University , AL-Beida, AL-Gabal AL-Akhdar region. It was envisaged that the different soil sterilized three methods such as Solar heating, Hot water and Flooding would effectively eliminate both fungi population and the damping off incidence which is probably made up of both pathogenic and nonpathogenic fungi. Fungal population was comparatively lower in soil treatment as compared to control. Lower fungal populations in soil treated plots as compared to non treated plots were reported by Venkateswarlu and Srinivasarao (2000) and Sharma *et al.* (1983). In the present study, results indicate that complete reduction in total count of all fungi was obtained with Solarization + transparent cover + Irrigation treatment. Similar result was obtained from Elabiedy and Ibrahim (2012). Transparent cover prevents the escape of long-waves radiation and water evaporation from the soil to the atmosphere, consequently exerting a greenhouse effect. In addition, the water vapours accumulated on the inner surface of the PE sheet further enhance the

greenhouse effect, resulting in higher soil temperatures (Stevens *et al.*, 1991; Elabiedy and Ibrahim 2012). Transparent cover prevents the escape of long-waves radiation and water evaporation from the soil to the atmosphere, consequently exerting a greenhouse effect. In addition, the water vapours accumulated on the inner surface of the PE sheet further enhance the greenhouse effect, resulting in higher soil temperatures (Stevens *et al.*, 1991; Elabiedy and Ibrahim 2012).

Also, Hot water treatment was more effective than other treatment. When soil were exposed to the lethal temperature (100°C), complete disinfestations was successfully achieved leading to the effective suppression of all fungi. This result attributed with Mahdy *et al.*, (2011).

In this study, *Fusarium* was found to be the most predominant fungus with the highest isolation frequency. *Fusarium* was detected to be predominant also in studies carried out in different soils, region and locations in the world (Ben-Yephet *et al.*, 1987, Saremi *et al.*, 2011; Elabiedy and Ibrahim 2012; oyeiyiola *et al.*, 2013).

Treatment	Fungi	Percentage %
Without treatment (Control)	<i>A. alternata</i>	7.04
	<i>A. niger</i>	7.04
	<i>F. oxysporum</i>	8.45
	<i>F. solani</i>	5.63
	<i>Mucor</i> sp	2.82
	<i>Phoma</i> sp	1.41
	<i>Pythium</i> sp	1.41
	<i>R. nigricans</i>	1.41
Solarization + Black cover	<i>T. viride</i>	4.22
	<i>U. botrytis</i>	4.22
	<i>F. oxysporum</i>	8.45
Solarization + Black cover + Irrigation	<i>F. solani</i>	5.63
	<i>T. viride</i>	4.22
	<i>F. oxysporum</i>	7.04
Solarization + Green cover	<i>A. niger</i>	2.82
	<i>P. digitatum</i>	2.82
	<i>F. oxysporum</i>	5.63
	<i>F. solani</i>	2.82
Solarization + Green cover + Irrigation	<i>C. fimeti</i>	1.41
	<i>T. viride</i>	4.22
	<i>F. oxysporum</i>	4.22
Solarization + transparent cover	<i>F. solani</i>	1.41
	<i>F. oxysporum</i>	4.22
Solarization + transparent cover + Irrigation	-	0.0
	-	0.0
Hot water	-	0.0
	-	0.0
Flooding	<i>F. oxysporum</i>	2.82
	<i>Penicillium</i> sp	1.41

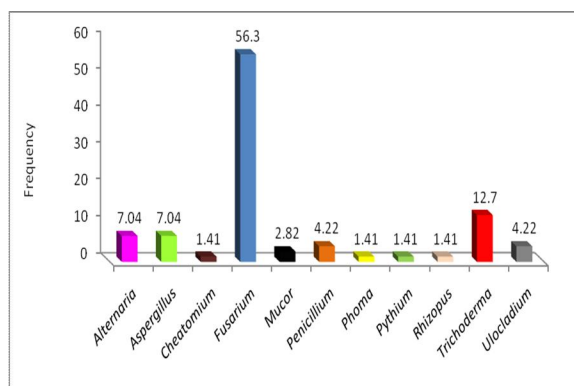


Plate 14. Frequency of fungi in soil pre- and post treatment

Under pot experiments, the highest reduction in Seedlings dying disease was obtained with hot water which reduced the disease incidence 0% for pre and post emergence respectively. Using of soil sterilization with hot water treatments for controlling several soil-borne diseases was reported about Fusarium wilt of spinach, (Kuniyasa *et al.*, 1993 and Iwamoto *et al.*, 2000); Fusarium wilt of Chrysanthemum (Iwamoto *et al.*, 2005) and Fusarium wilt of melonis (Ogawara, *et al.*, 2006). The hot water treatment is easier to use than steam sterilization and,

unlike solar heat sterilization, its application is not limited to the summer season. Because the wet-heat provided by the hot water does not wipe all the living organisms out, this technology is regarded as an eco-friendly Methyl bromide alternative that can widely be applied to various crop production (Noling, 1995 and Kita, *et al.*, 2003).

Fusarium spp, *Trichoderma*, *Alternaria*, *Aspergillus* and *Penicillium* spp, their common occurrence could also be due to their high sporulating nature and this is also coupled with their ability to grow well on laboratory media (Oyeyiola and Hussein, 1992; El-Said and Saleem, 2008). This finding is in consonance with the work done by Ekundayo, (2004).

Frequency of *Fusarium* sp recorded in most treatments due to the genus *Fusarium* from adamant fungi that tolerate the highest temperature and produce chlamydo spores which survival in soil for long period (Ben-Yephet *et al.*, 1987). In this respect Kita *et al.*, (2003) reported that in Japan, when hot water is applied onto the soil, the surface is immediately heated but soon cools down whereas the temperature of the deeper soil gradually increases and remains high for few days after the treatment. In the 30cm depth, temperature over 55°C, the lethal temperature for the *Fusarium*. Actually, when *Fusarium oxysporum* f.sp. *lycopersici* present within the 30cm depth soil was exposed to the lethal temperature, complete disinfestations was successfully achieved leading to the effective suppression of the wilt disease

Treatments	Seeds decay %	Seedlings dying %	Seedlings survival %
Control	10 (18.44) ab	15 (22.79) bc	75 (57.42) b
Solarization + Black cover	00 (00.00) b	25 (30.00) ab	75 (57.42) b
Solarization + Black cover + Irrigation	10 (18.44) ab	10 (18.44) bc	80 (63.44) b
Solarization + Green cover	00 (00.00) b	25 (30.00) ab	75 (60.00) b
Solarization + Green cover + Irrigation	15 (22.79) a	5.0 (12.92) cd	80 (63.44) b
Solarization + transparent cover	5.0 (12.92) ab	10 (18.44) bc	85 (67.21) b
Solarization + transparent cover + Irrigation	5.0 (12.92) b	5.0 (12.92) bc	90 (71.56) b
Hot water	00 (00.00) b	00 (00.00) d	100 (90.00) a
Flooding	10 (18.44) ab	10 (18.44) bc	80 (63.44) b

Means of the same column followed by the same letter do not differ according to Duncan's multiple range test ($P = 0.05$)
Value in the parentheses is angular transformations
Each treatment represented by 10 seeds for each pot were used.

Table 4. Effect of soil sterilization on Disease severity with damping off.

Treatments	Disease severity
Control	0.39 a
Solarization + Black cover	0.13 b
Solarization + Black cover + Irrigation	0.05 b
Solarization + Green cover	0.13 b
Solarization + Green cover + Irrigation	0.14 b
Solarization + transparent cover	0.12 b
Solarization + transparent cover + Irrigation	0.12 b
Hot water	0.0 b
Flooding	0.1 b

Means of the same column followed by the same letter do not differ according to Duncan's multiple range test ($P = 0.05$)

Conclusion:

From this study it can be concluded that irrigation + transparent and hot water have better impact on the fungal population of the soil than other treatment. The application of two methods at the same time to control soil-borne fungi in future.

Data in Table (4) show that all sterilization treatments decrease the disease severity from 0.39 in control to 0.0 in hot water treatment. Results agree with Dabag (2001) who reported that solar heating was affected to control soil born fungi, Nematodes and weeds, then increase and enhancing the plant growth.

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