

Increasing of Tomato Yield Grown in Hydroponic System Using *Pythium oligandrum* Isolated from Khoaa, Aljouf, Saudi Arabia

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THERE WERE excellent results for tomato yield in hydroponic applications in many countries of the world. It is desirable to look for ways designed to test the ability of two isolates of *Pythium oligandrum*, isolated from rhizosphere of alfalfa cultivated in Khoaa, Sakaka, Saudi Arabia, to increase yield of tomato in hydroponic system. There were significant increase of fresh and dry matter of shoot, root systems as well fruits of tomato plants cultivated in hydroponic system supplemented with each of *P. oligandrum* isolates (JU0328 & JU0329). Results showed that hydroponics cultivation system treated with *P. oligandrum* had beneficial effect on increasing yield of tomato as well as suggesting alternative methods to traditional agriculture, especially in arid areas with poor soil quality.

Keywords: Biofertilizers, Hydroponic, *Pythium oligandrum*, Saudi Arabia, Tomato.

Introduction

In places characterized by types of arid mechanically and chemically poor soil, aquaculture methods may be a promising way to grow some crops.

The production systems of hydroponics range from those that have sophisticated infrastructure to others that require regular plastic huts with limited possibilities to small operations in standard rooms or on roofs.

Literature records several investigations for aquaculture applications in many countries of the world (Victor, 2017). Therefore, it is important to seek ways to increase production of hydroponics for important crops used extensively moreover it is desirable that these methods are considered safe from environmental and health perspectives. Organic fertilizers such as adding useful types of certain fungi has been found to increase productivity (Kaewchai et al., 2009). Fungi such as isolates of *Trichoderma* and *Pythium* were used as biofertilizers to increase crop productivity mainly in ordinary solid soil (MrBill, 2016 and Darren, 2017). *Pythium oligandrum* Drechsler

has been recorded for increasing yield in ordinary soil (Abdelzاهر et al., 2015). On the other hand, unfortunately the use of *P. oligandrum* in hydroponics has not been adequately studies.

In many areas of Saudi Arabia, such as the northern region of Aljouf, Sakaka, soil is characterized by organic poverty and mechanical poorness. Therefore, we elected to work on the development of hydroponics as a mean of agricultural production of important crops such as tomatoes.

In a previous study, *P. oligandrum* was isolated in a survey of pythiacous fungi of some locations in Saudi Arabia (Abdelzاهر et al., 2015).

In Saudi Arabia, ability of the isolated *P. oligandrum* to increase the productivity of some plants has not been tested. As is known from many previous studies, *P. oligandrum* can be used to control plant diseases as well as to increase productivity (Elnaghy et al., 2014a). In this regard, with the success of *P. oligandrum*, it has been commercially produced for use in biological control and increasing plant production. Biocontrol application by adding dried granular

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of *P. oligandrum* oospores or its powder growth to the agricultural soil was reported. Polygangron® by Vyskumny Ustav of Slovak Republic is one of the famous commercial brand of *P. oligandrum* to be used in soil agriculture (Khetan, 2001). Studies have shown that *P. oligandrum* secrete auxin-like substances on and inside roots of the target plants that have been invaded (Le Floch et al., 2003). Use of commercial *P. oligandrum* in aquaculture has not been adequately studied for crop application.

This study concern to introduce a persuasion of aquaculture to farmers in the northern region of Saudi Arabia, especially in the vicinity of the city of Sakaka in Aljouf region.

One of the objectives of this study was to isolate *P. oligandrum* from Khoaa Village of Sakaka in Aljouf region in the north of Saudi Arabia and test the ability of two selected isolates to increase the growth of tomato seeds (in water agar medium) as an indicator of their positive use in the present experiment. The other objective was to study the ability of the two selected strongest isolates of *P. oligandrum* to increase the production of tomato fruits grown in aquaculture.

Materials and Methods

Sampling locations

Fifty samples were collected in February 2016 from rhizosphere of Alfalfa cultivated near the village of Khoaa in Aljouf area in the north of Saudi Arabia, (coordinates 29° 48' 6" N, 40° 26' 27" E).

Isolation of Pythium spp.

Dilutions were made from alfalfa rhizosphere soil (5 gm) using sterilized distilled water from 10^{-1} to 10^{-5} . One ml of each diluted soil was poured on NARM selective medium in Petri-dishes [Nystatin (10 mg/L), Ampicillin (250 mg/L), Rifampicin (10 mg/L) and Miconazole (1 mg/L) in corn meal agar (CMA, CMA; 17 g/L, BD-BBL™) (Elnaghy et al., 2014b)]. Incubation of the inoculated dishes was at 27°C for 3 days or until emergence of pythiaceus colonies.

Identification of the isolated Pythium spp.

Identification was performed using morphological criteria [original description of the species, and different successive historical keys for identification (Drechsler, 1930, Middelton, 1943, Waterhouse, 1968, Plaats-Niterink, 1981, and Dick, 1990)]. Identification was confirmed by molecular criteria [using nuclear rDNA region of the internal transcribed spacer (ITS), including

5.8S of rDNA], Kageyama et al. (2003).

Selection the most virulent isolates of P. oligandrum

Nineteen isolates of *P. oligandrum* were inoculated on CMA medium and incubated at 27°C until mycelia growth filled the whole dish. Tomato seeds (which have been tested for viability by test the ability of seeds to produce plumages and radicles) were placed on the fungal growth and then incubated in a growth chamber at 25°C (ideal temperature for germination of tomato seeds) with illumination of 12 h photoperiod ($91 \mu\text{mol m}^{-2} \text{S}^{-1}$). After five days, lengths of radicles and plumages of tomato germinating seeds were measured (using thread and ruler). It is assumed that strongest isolates of *P. oligandrum* give longest growth of tomato plantlets.

Preparing of P. oligandrum inoculum

P. oligandrum was grown on V8 broth medium supplemented with wheat germ oil at 27°C for 10 days in shaking incubator in the dark. Each 300 ml Erlenmeyer flask containing 100 ml V8 broth medium with wheat germ oil (1 ml per liter) was inoculated with 4 mycelial discs of the actively growing growth of *P. oligandrum* previously cultivated on PDA medium. After harvesting mycelia pellets, it was homogenized together with distilled water (3 pellets + 100 ml distilled water) and either used directly or was kept in a refrigerator to be used in a due course (not exceed 3 days).

Plant culture

Effect of root colonization by P. oligandrum and produced yield of tomato cultivated in hydroponic cultures

A tomato variety that has unlimited vegetative growth should be selected as the plant ends with a vegetative bud that continues to grow upward, leading to increased plant height. Accordingly, tomato seeds (cv. Hildares) were surface sterilized by immersed it in 70% ethanol for 5 min, soaked in 5% aqueous sodium hypochlorite for one minute and carefully rinsed 3 times in sterile distilled water. These seeds were then sown in boxes of white cork (100x50x20 cm) and fertilized daily with a nutrient solution containing in meq. 14.5 NO_3^- ; 1.8 H_2PO_4^- ; 7.5 K; 8.8 Ca; 4.0 Mg and 0.5 NH_4^+ until the shoot system of plants reaches about 5 cm. After removing the plants from the pebble soil, each seedling was replanted in pots containing fine rocks and left for 7 days in a growth chamber at 25°C (with illumination of 12 h photoperiod ($91 \mu\text{mol m}^{-2} \text{S}^{-1}$)). Seedlings were removed carefully from pots and their roots soaked in a 3% carboxy methyl cellulose (CMC) + solution containing

previously papered homogenized solution of mycelia and oospores of *P. oligandrum* and left for 30 min at room temperature.

Control samples were only soaked in CMC without the fungus. Each tomato seedling was transplanted in a bucket containing the nutritious solution so that the plant root system positioned within the solution and the shoot system was fixed by a longitudinal cord to the top of the greenhouse, height of two meters. Buckets bottom were covered with black plastic bags, to prevent light from reaching the root. In order to aerate roots of tomatoes and supply them with oxygen, each bucket was connected to an air compressor source. Level of the nutritious solution in every bucket was followed up and the appropriate amount was, subsequently, added to the desired level. During the flowering period of tomato plants, pollination process is carried out by treating floral swings to vibrations using an electric toothbrush to distribute pollen grains on stigma. This process is performed every two days throughout the flowering period. The experiment was performed in a greenhouse under a 14-h-light 10-h-dark photoperiod. Plants were regularly fed with nutrients stated in the following Table (Passam et al., 2007).

Nutrient solution in hydroponic system for growing of tomato plants.

Factors & elements	Starter	Re-filling
EC. dS m ⁻¹	3.7	1.5
pH	5.5	5.5
Solution A (mmol/l)		
NO ₃ -N	23	10.8
NH ₄ -N	0.1	1.0
K	8	6.5
P	1.0	1.25
Ca	10	2.75
Mg	4.5	1.0
S	6.8	1.5
Solution B (μmol/l)		
Mn	5	10
Mo	0.5	0.5
B	50	20
Fe	25	15
Zn	7	4
Cu	0.75	0.75

The pH of the nutrient solution and the greenhouse temperature were regularly monitored and ranged from 5.5 to 6 and 18 to 25°C, respectively. Plants were harvested after 8 weeks, shoot and root fresh and dry weights (FW) and

(DM) were calculated. Fruit fresh and dry weights (FW) were also calculated.

Statistical analyses

(two-way ANOVA; P = 0.05; n = 7) were used (Gelman, 2005).

Measurement of *P. oligandrum*-root colonization

Roots of the cultivated tomatoes either from control or from *P. oligandrum*-inoculated plants were collected every 14 days from buckets. Root system of tomato plant was washed thoroughly by placing it in a closed sieve mesh and leaving it under tap water for 15 min (Abdelzaher, 2013). Drain the washed roots between folds of sterile filter paper and then re-wash with sterile distilled water and dry again between sterile paper filter folds. Five-mm segments from each of the tap roots, lateral roots and root hairs were cut and cultured onto NARM selective *Pythium* isolation medium and then incubated at 25°C in the dark. Fifty root segments were cultured per tomato plant and six tomato plants in six pots from six randomly selected rows were used in this test. After appearance *P. oligandrum* colonies, identification was confirmed and growth was counted, and data were expressed as the percentage of root pieces from which *P. oligandrum* were covered.

Results

Fifty rhizosphere samples from alfalfa plants were collected from a field located in Khoaa Village, Sakaka City, Aljouf region, Saudi Arabia (Fig.1).

Isolation and identification of *Pythium* spp.

Nineteen isolates of *P. oligandrum* out of 69 *Pythium* spp. were isolated from rhizosphere of alfalfa using dilution plate method via NARM selective medium for isolation of pythiaceus fungi.

Identification was done using keys for identification of *Pythium* spp. following criteria of zoosporangia, antheridia & oogonia and oospores (Drechsler, 1930; Middleton, 1943; Waterhouse, 1968; Plaats-Niterink, 1981 and Dick, 1990). Confirmation of the identification of *P. oligandrum* was performed by sequencing the nuclear rDNA region of ITS, including 5.8S of rDNA which gave sequences identical (100%) to *P. oligandrum* in the (Genbank accession number, AY986954.1) (Fig. 2). Although *P. hydnosporum* and *P. amasculinum* have the same sequence, they are clearly different from *P. oligandrum* on the basis of asexual and sexual structures.

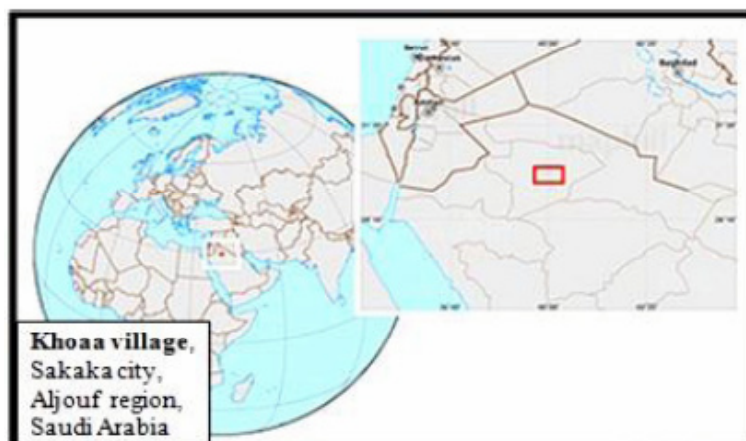


Fig. 1. A map of Saudi Arabia, shows location of sampling site of Khoaa Village (<http://www.maphill.com/saudi-arabia/jawf/sakaka/location-maps/gray-map/free>).

CCACACCTAAAACTTCCACGTGAACCGTTATAACTATGTTCTGTGCTTCGTCGCAAGAC
 TTGAGGCTGAACGAAGGTGAGTCTGCGTCTATTTGGATGCGGATTTGCTGATGTTATTTT
 AAACACGTATTACTTAATACTGAACTATACTCCGAATACGAAAGTTTTTGGTTTTAAACAAT
 TAACAACCTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGC
 GATACGTAATGCGAATTGCAGAATTCAGTGAGTCATCGAAATTTGAACGCATATTGCACT
 TTCGGGTTATGCCTGGAAGTATGCCTGTATCAGTGTCCGTACATCAAACCTTGCTTTCTTTT
 TTTGTGTAGTCAAAATTAGAGATGGCAGAATGTGAGGTGTCTCGCGTTGTCTTTTAAAGA
 TGGTTCGAGTCCCTTTAAATGTACGTTGATTCTTTCTGTGTCTGCGAATTGCGATGCTATG
 CTCTTTGTGATCGGTTTAGATTGCTTTGCGCTGGTGGGCGACTTCGGTTAGGACATATGGA
 AGCAACCTCAATTGGCGGTATGTTCCGGCTTGCCTGACGTTTCAGCTAAGCGAGTGTGGTTT
 TCTGTCTTTCCCTTGAGGTGTACCTGTGCTGTGAGGTTGATTTAGGCTATATGGTTGCTT
 GTTGTGTGGTTATCGTTTTTCAGACGCCTGCTTCGGTAGGTAAAGGAGACAACACCAATT
 TGGACTGAGAGTTTACTCTTTTTTCACT

Fig. 2. Sequencing of nitrogen bases of nuclear rDNA region of ITS, including 5.8S of rDNA of one representative isolate of *P. oligandrum*.

Selection of the the most virulent isolates of *P. oligandrum*

Only two isolates of *P. oligandrum* gave longest plantlet (radical + plumage) of tomatoes planted in WA medium covered with grown fungus were selected to perform the rest of experiments. Isolates of JU0328 and JU0329 were selected on this basis.

Assessment of root colonization by *P. oligandrum*

Data in Table 1 shows significant colonization of tomato roots by the two isolates of *P. oligandrum*. Obviously, colonization of tomato roots by *P. oligandrum* was stable over the whole development period. The fungus was emerged from about 35 to 50% of the collected roots sampled during the test.

Effect of root colonization by *P. oligandrum* and yield of tomato plants cultivated in hydroponic cultures

Results showed significant increase of fresh and dry weight of shoot, root systems and fruits

fresh and dry matter of tomato plants cultivated in hydroponic system supplemented with each of the *P. oligandrum* isolates (JU0328 & JU0329). It was shown that isolate JU0328 was more effective than the second one (Fig. 3-5).

Discussion

Beneficial *Pythium* spp. are widespread in all soil types in many countries but are abundant in clay ones especially in the rhizosphere (Elnaghy et al., 2014b). Since the first identification of *P. oligandrum* by Drechsler in (1930), it has been isolated from many regions of the world (Kinoshita & Ichitani, 1996; Abdelzaher et al., 1997; Abdelzaher, 2013; Elnaghy et al., 2014b and Abdelzaher et al., 2015). Studies conducted on *P. oligandrum*, revealed its activity to protect against many plant pathogenic fungi. Scientists found that this fungus has a positive activity to increase growth of crop plants and thus increase the yield (Benhamou et al., 2012).

TABLE 1. Root colonization by two isolates of *P. oligandrum* of tomato plants (cv. Hildares) grown in hydroponic greenhouse irrigated with nutrient solution (Passam et al., 2007). Data are expressed in percentage of colonized roots (50 roots per plant X 6 samples = 300).

Days of tomato plants growth in nutrient isolation	Frequency of <i>P. oligandrum</i> (%)	
	JU0328	JU0329
14	35	36
28	40	38
42	42	44
56	48	48

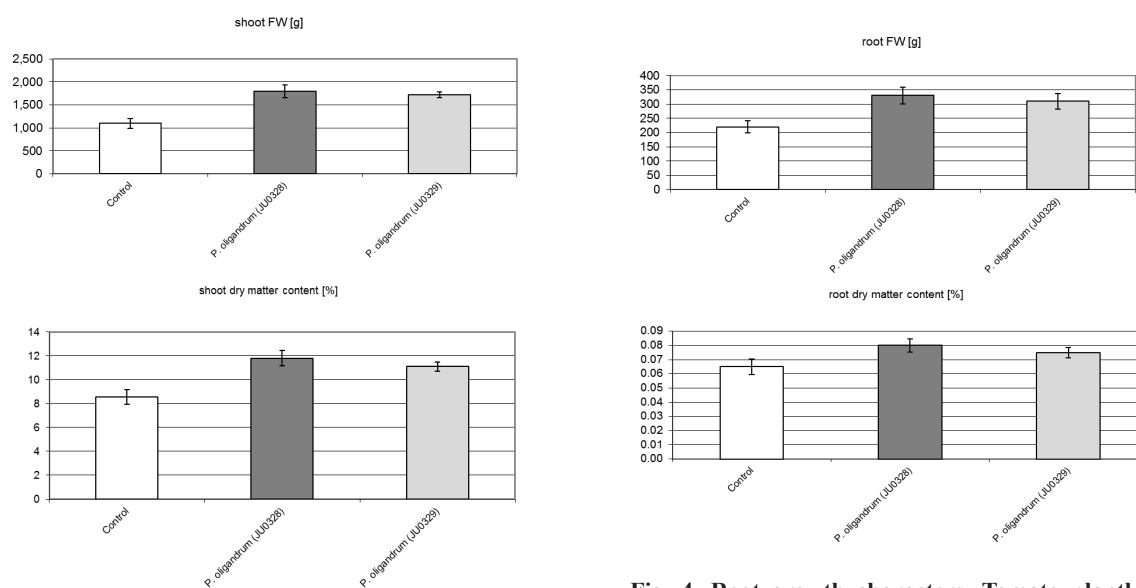


Fig. 3. Shoot growth characters. Tomato plantlets were not inoculated (Control), inoculated with two isolates of *Pythium oligandrum* (JU0328 & JU0329) singly and cultivated in hydroponic cultures. Two-way ANOVA; $P = 0.05$; $n = 7$ showed significant influence of the factors of both isolates of *P. oligandrum* on fresh shoot and dry matter. There were significant differences between each treatment and the control.

Many previous studies have proven beyond doubt the ability of *P. oligandrum* to produce plant-like auxins that accelerate vegetative growth of plants (There were no experiments on the production of these hormones in this research), (Le Floch et al., 2003). It is known that there are significant disparities between isolates of the same species in terms of physiological effects of these organisms. From this point of view, this research was designed to obtain growth promoting isolates of *P. oligandrum* and use their beneficial ability to increase the productivity of tomato crop in aquaculture. In this study, 19 isolates of *P.*

Fig. 4. Root growth characters. Tomato plantlets were not inoculated (Control), inoculated with two isolates of *Pythium oligandrum* (JU0328 & JU0329) singly and cultivated in hydroponic cultures. Two-way ANOVA; $P = 0.05$; $n = 7$ detected significant influence of the factors of both isolates of *P. oligandrum* on fresh and dry root. There were significant differences between each treatment and the control.

oligandrum were obtained from rhizosphere of cultivated alfalfa plant in an area where these fungi were previously isolated (Abdelzaher et al., 2015). Two of these 19 isolates were selected, based on enhanced seedling length of germinating tomato seeds, to test their influence on increasing growth and productivity of tomatoes in aquaculture. It is worth to mention that our morphological identification of the isolated *P. oligandrum* was meeting with the molecular confirmation. This can be attributed to ease and clarity of identification criteria as well as the characteristic shape of zoosporangia which resemble fruits of peanuts and the thorn-form oogonia (Abdelzaher, 1999).

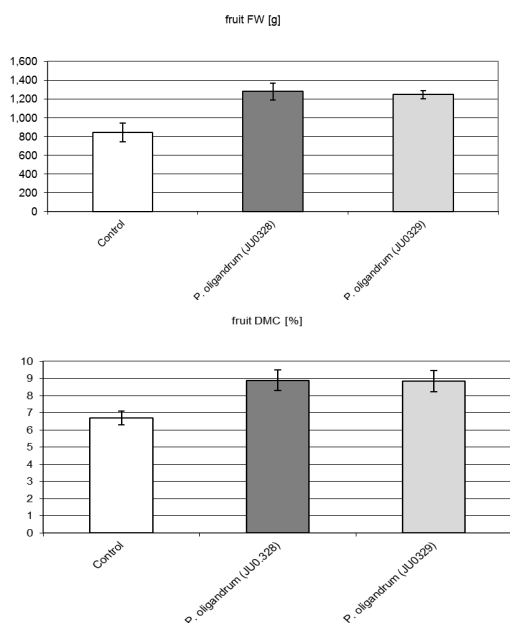


Fig. 5. Fruit biomass. Tomato plantlets were not inoculated (Control), and inoculated with two isolates of *Pythium oligandrum* (JU0328 & JU0329) and cultivated in hydroponic cultures. Two-way ANOVA; $P = 0.05$; $n = 7$) showed significant influence of the two isolates of *P. oligandrum* on fruit FW and DW.

Results of this study showed ability of the two isolates of *P. oligandrum* to settle on and within roots of tomato plants grown in the hydroponic. Fungi were re-isolated from 35% to 50% of the tested root samples and this is largely consistent with many previous studies (Le Floch et al., 2005). As a result of presence of *P. oligandrum* in the tissues of roots of tomato plants, the fungus which is known to secrete plant-like auxins, can increase growth and yield of plants as compared to control samples (Le Floch et al., 2003 and Elnaghy et al., 2014a). This phenomenon was confirmed from previous studies and has not been addressed in this study.

Results of the experiments in this study showed beyond any doubt the ability of the two isolates of *P. oligandrum* to increase growth and yield of tomato (cv. Hildares) as fresh and dry of root, shoot and fruits weights of tomato plants cultivated using hydroponic methods. Fresh root weights and dry ratio in fungal treated samples were increased by 33 and 19% as compared with control samples, respectively. Similarly, fresh shoot weights and dry ratio in fungal-treated samples were increased by 39 and 33%

as compared with control samples, respectively. As a result, biomass of tomato fruits as fresh weights and dry ratio in fungal treated samples were increased by 32 and 22% as compared with control samples, respectively. The first isolate (JU0328) showed a greater effect on tomato plants than the second (JU0329) one, but with slight differences. This is in harmony with many previous research (Hase et al., 2012, Benhamou et al., 2012 and Elnaghy et al., 2014b) but it is worth mentioning that fungal isolates used here showed a strong effect by increasing the proportion of soft fruits of tomato by more than 30% compared with samples that were not treated with the fungus. This leads to the possibility to search for new even stronger isolates of *P. oligandrum* due to the apparent difference in the effect of these fungi on increase of yield of tomato crop. Results of this study show a way to expand use of hydroponics to be easier treated with *P. oligandrum* and to find alternative methods to traditional agriculture, especially in arid areas with poor soil quality.

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زيادة غلة الطماطم في الزراعة المائية باستخدام فطر *بيثيم أوليقاندرم* المنعزل من خواء، الجوف، المملكة العربية

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والميكروبيولوجيا - كلية العلوم - جامعة المنيا - المنيا- مصر.

أجريت التجارب لإختبار قدرة عزلتين من فطر *Pythium oligandrum*، معزولة من ريزوسفير نبات البرسيم المنزوع في قرية خوعاء، سكاكا، المملكة العربية السعودية، لزيادة غلة الطماطم في نظام الزراعة المائية. و أظهرت النتائج زيادة معنوية في إنتاج الطماطم المنزوعة في نظام الزراعة المائية المدعم من كل من عزلتي فطر *بيثيم أوليقاندرم* المختبرتين . (JU0328 & JU0329). كما أوضحت نتائج هذه الدراسة طريقة التوسع في الزراعة المائية المدعمة بفطريات *بيثيم أوليقاندرم*، النافعة كمكافح بيولوجي و مدعم لنمو النبات من أجل زيادة غلة الطماطم باستخدام طريقة بديلة للزراعة التقليدية، وخاصة في المناطق القاحلة ذات نوعية التربة الرديئة.