



EFFICIENCY OF SOME BIOLOGICAL CONTROL AGENTS AND PLANT EXTRACTS AGAINST *FUSARIUM SOLANI* CAUSING AGENT OF DAMPING OFF DISEASE ON TOMATO

Fatima Hadi Kareem and Ahed A.H. Matloob

Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.
ayadmj77@gmail.com

Abstract

The aim of this study was to isolate and diagnose the causing of Damping off Disease on Tomato in the Babylon governorate-Iraq. And evaluate the effectiveness of *Trichoderma harzianum* and some plant extracts in the control of the pathogen. The results of isolating the fungus from the affected plant parts of the tomato seedlings revealed the emergence of 3 isolates of *Fusarium solani*. The results of the test of pathogenicity showed that all isolates of the fungus had a pathogenic ability. The results showed that *T. harzianum* was effective against the *F. solani* and with a high inhibitory rate. The bacteria *Bacillus thuringiensis* were low effective inhibition of pathogen growth. The water extract of the leaves of the *Conocarpus lancifolius* and the *Xanthium strumarium* (Rough cocklebur) (5, 10 and 15%) was effective in the fungus *F. solani* (Fs3) and with a significant superiority of the Rough cocklebur extract at 15% concentration. The results showed that the agents used in the study provided good protection for tomato seed and seedlings from infection. The treatment of interference between *T. harzianum* and Bacteria and the Rough cocklebur extract was protected the seeds and improved their germination to be 88.88% compared to the germination rate of the pathogen, which had a germination rate of 33.33%. The integrated treatment between the *T. harzianum*, *B. thuringiensis* and Rough cocklebur extract was superior in reducing the disease incidence and severity of infection into 25.92% and 13.33% respectively and close to the treatment of chemical fungicide Beltanol, which was the 14.81 and 8.14%, respectively. And improved the parameters of plant growth which included length of plants and wet and dry weights under Nursery condition.

Keywords : Tomato, Damping off, *Fusarium solani*, *Trichoderma harzianum*, Beltanol, plant extract.

Introduction

Tomato (*Lycopersicon esculentum* mill) is one of the main and essential vegetable crops, the area cultivated in Iraq in 2017 (55515) donum and a production of (304921) tons (Central statistical origination, 2017). Tomato is infected by many pests, such as insects, fungal and viral diseases. These diseases include Damping off and root rot, this disease is one of the most important diseases of nurseries and fields. It is widespread all over the world (Agrios, 2005; Larousse *et al.*, 2017; Ahlem, 2018). This disease caused by many soil borne fungi, The severity of this disease is associated with the high and low soil temperature, moisture and delayed seed germination. Because these organisms live in the soil, they often destroy the root mass without feeling their presence (Garret, 1977; Noe and Campbell, 1985). *Fusarium solani* is one of the most important and common soil borne fungi causing agent of many diseases. The amount of loss due to this disease is significantly associated with the intensity of the pathogenic fungi inoculum available in the soil and the planting season and the presence of biological agents (Mazzola, 1996; Shenashen *et al.*, 2017). The damping off disease affects the seeds and seedling of most vegetable crops, whether planted directly in the field or planted in the nursery causing significant losses through the death or rot of seeds before germination or after germination or the death of young seedlings pre and/or post emergence and rot root, so farmers to use Large quantities of seeds to overcome the problem of seed germination failure or re-cultivation (Harikrishnan *et al.*, 2002). The recent trend in the control of various agricultural pests tends to use integrated pest management to reduce the use of chemical pesticides and reduce their environmental and economic disadvantages. Most researchers have now sought to use various possible methods to keep away from the use of chemical pesticides

such as biological agents as a practical and safe solution to disease control Especially root diseases such as damping off and root rot because of problems in which many complications (Papavizas and Lumsden, 1980). The biological control agent *Trichoderma* spp., especially *T. harzianum* has a high antagonistic properties. This is one of the most well-known species in control to the remaining species (Howell, 2006; Siddiquee *et al.*, 2007; Ulker *et al.*, 2011). Other factors used in the control of fungus *F. solani* are Plant Growth Promoters Rhizobacteria (PGPR). These bacteria are known for their high efficiency in nitrogen fixation and their activity to control with many pathogens (EL-Komy, 2001; Brasileiro *et al.*, 2004). The researchers' interest in the use of plant extracts in the control of many plant pathogenic fungi has been enhanced by the fact that these extracts contain effective secondary metabolites with environmentally desirable properties such as rapid degradation, low toxicity, and high specialization (Lokendra, Sharma, 1987; Singh *et al.*, 1980; Bonanomi *et al.*, 2011). Due to the importance of damping off and root rot disease on the tomato caused by *F. solani* and the need for more necessary information to control the disease and reduce the economic damage of the use fungicides and given the lack of studies on the control of the disease with the use of *T. harzianum* and plant extracts and compare with a chemical pesticide was aimed in This study is based on the Isolation and diagnosis of the cause of damping off and rot root disease of the tomato from some fields of the province of Babylon. Evaluation of the effectiveness of the biological control *T. harzianum* and some plant extracts against *F. solani*.

Materials and Methods

Sampling and isolating *Fusarium solani* from infected seedlings and tomato plants

Infected pieces from stem and root regions of diseased tomato plants showing root rot symptoms were collected separately from agricultural field of Babylon province, Iraq. Tissue pieces were surface sterilized with 1% sodium hypochlorite for two minutes and subsequently washed twice with sterile distilled water. They were placed on potato dextrose agar (PDA) medium and incubated in incubator at 25 ± 1 °C for 5 days. The culture was identified based on morphological characters (Booth, 1971). The *F. solani* was purified transferring the pieces of the mycelia into PDA medium.

Effect of *Fusarium solani* isolates in the germination of tomato seeds

The Pathogenicity of three isolates of *F. solani* (Fs1, Fs2, Fs3) obtained by isolating from infected tomato plants was tested and the method previously described by Bolkan and Butler (1974) was adjusted with some modification and using local tomato seeds (Non- fungicides), with 9 cm diameter Petri dishes containing 20-15 ml of water Ager medium (20 g agar in 1 liter distilled water) and sterilizer with a temperature of 121 °C and 1.5 kg / cm² For 15 minutes, the tetracycline antibiotic is added, then the dishes are left at laboratory temperature to harden and then the dishes are inoculated by 0.5 cm diameter piece of *F. solani*, in the center of the dish. This test was performed for isolates individually. The dishes Incubated at 25 ± 1 °C for 48 hours and then sow the seeds of a local dish in the dishes prepared after surface sterilization with sodium hypochlorite solution (1% free chlorine). The seeds were distributed in a circular manner parallel to the edge of the dish, the dishes were placed in the incubator at 25 ± 1 °C for 7 days. The results were then calculated by counting the number of germinated seeds and the germination rate, according to the following Formula: % Seed germination = Number of seeds grown /total number of seeds×100.

Test of the antagonistic potential of *Trichoderma harzianum* against *Fusarium solani* on PDA medium

T. harzianum were obtained from the Plant Pathology Laboratory/Al-Mussaib Technical college. Its antagonistic potential against pathogenic isolate of *F. solani* was tested in a double culture method, The dishes were placed in an incubator at 25 ± 1 °C for one week. The Bell *et al.* (1982) 5-degree scale was used to estimated antagonistic potential which include: 1. *Trichoderma* cover the entire area of the dish without allowing pathogenic fungi to grow. 2. *Trichoderma* cover 3/4 of the area of the dish while the pathogen cover the remaining area of the dish. 3. *Trichoderma* cover half the area of the dish and the fungus covers the other half of the dish. 4 - The biological fungus covers 1/4 of the area of the dish, while the pathogenic fungus covers the remaining 3/4 of the dish. 5. the pathogenic fungus cover the entire area of dish. A biological agent is effective in terms of control when showing a degree of contrast (2) or less with pathogenic fungus.

Efficiency of *Bacillus thuringiensis* (Bt) against *Fusarium solani*

Bacteria *B. thuringiensis* (Bt) were obtained from the biological control Laboratory. The required amount of

bacterial inoculum was prepared for use in the experiments by growing the bacteria on the Nutrient Broth medium where 100 mL of this medium was placed in a 250 mL conical flask and inoculated with bacteria taken from 2-day bacterium colony age and incubated at 28 ± 1 °C for 3 days. The dishes containing the PDA medium were then inoculated by taking 1 ml of the bacterial suspension by using a sterile pipette and placed in the dish. and placed in the center of the dish 0.5 cm from the *F. solani*. The control treatment was prepared with the *F. solani* alone. After that, the dishes were placed in an incubator for 7 days, The fungus growth was calculated by calculating the colony diameter of the pathogenic fungus The percentage of inhibition was calculated according to the following equation:

$$I = \left(\frac{R-r}{R} \right) \times 100$$

where I=Percentage of inhibition

R=colony diameter in control

r=colony diameter in treatment

Plant extracts

Two plants were selected to study their effect against *F. solani*. The leaves of the *Conocarpus lancifolius* and the *Xanthium strumarium* (Rough cocklebur) were included, Plants were collected from some fields and gardens in Babylon Governorate/Al-Mussaib Technical college and Al-Mussaib Technical Institute for 2017-2018 season. The plant samples were then dried by brushing them in the form of thin layers over wide surfaces of cloth and exposing them to the sun for a suitable period of time, while stirring continuous samples to prevent them from rotting and accelerating drying. Plant samples were crushed using an electric blender. Place each plant powder in polyethylene bags with the name of the plant and the weight, and stored in the refrigerator until use.

Test the effect of water extract of some plants in the growth of *F. solani*

The extraction was done using the method of Shekhawat and Prasad (1971), where a certain weight was taken from each of the used plants (*Conocarpus* and Rough cocklebur) and placed in a 250 mL glass flask and added to distilled water (1:10) the flask put in electric shaker for 24-hour. The solution was Filtered with a clean, sterile cloth to dispose of the large particles. Concentrate the total solution from the extraction into a water bath at 45 °C to get rid of the water and obtain a thick liquid. The extracts were weighed and stored in glass bottles marked and sealed and placed in the refrigerator until use. The efficacy of the different plant extracts was tested using the food poisoning method, taking 5, 10 and 15 ml of concentrated solution and added to 95, 90, 85 ml of sterile PDA to obtain a concentration of 5, 10 and 15%, respectively. Dishes filled with treated media. After the hardening of the medium, the dishes were inoculated in the center with a 0.5 cm diameter disc from the *F. solani* colony at 5 days age. The results were calculated by estimated the rate of measurement of diameter from of each colony and the percentage of inhibition was calculated as use the following formula:

$$\% \text{ Inhibition} = \left[\frac{R - r}{R} \right] \times 100$$

where,

r = the radius of the fungal colony in treatment,

R = the radius of the fungal colony in control.

The effect of some biological and Plant Extract in controlling Damping off Disease on tomato seedlings and their effect on some growth parameters under on the nursery conditions.

This experiment was conducted in the plastic house of the Department of Biological control Techniques- Al-Mussaib Technical college for the spring season 2018. Styropor, 11 X 19 holes plates were used and the treatment were added to the experimental units which included to: 1- *F.solani* alone (Fs-3). 2- Fs-3+*T. harzianum* (Th). 3-Fs-3 + *Bacillus thuringiensis* (Bt). 4- Fs-3+ Rough cocklebur Extract (RE). 5- Fs-3 + Th+ Bt. 6- Fs-3 + Th+ RE. 7- Fs-3+Bt+RE. 8- Fs-3 + Th+ Bt + RE. 9- Fs-3 + Beltanol. 10-control. 11- Th(alone). 12- Bt (alone) .13- RE (alone). 14- Th + Bt. 15- Th + RE. 16- Bt+RE. 17- Th+ Bt +RE. The *F. solani* that grown on the PDA medium by 3 pieces diameter 1 cm² / pore. A 15 mL/Rough cocklebur Extract was added to the plant, The Beltanol at concentration of 1 ml / liter was added after a day of adding the fungus. *T. harzianum* was added a week ago from soil contamination with pathogenic fungus with an average of 3 pieces of diameter 1 cm²/pore was taken from a 7-day colony. And *B. thuringiensis* was added by 15 ml / plant and a 3 day old colony growing on the liquid N.B medium a week before contamination with pathogenic fungus. Control treatment was applied and without any addition, each treatment was repeated 3 times and each repeater included 3 pores. The dishes were sown with 3 tomato seeds/pore. The percentage of germination was calculated after the germination of the control seeds was completed. At the end of the experiment (after 30 days), two plants were removed from each experimental unit and randomly. The roots were then washed thoroughly with water to remove the soil and the Disease incidence and severity of the root rot disease was determined and length of plant and wet and dry weight. the disease incidence of root rot per field was estimated using the following formula:

Disease incidence(%) = number of infected plants/total number of tested plants ×100. and the severity of the root mass were calculated according to the 5-point disease index as follows: 0 = healthy roots. 1 = Secondary root is rot. 2 = Secondary root is rot and part of the main root. 3 = Root rot without rotting crown. 4 = Root rot and rot of crown. 5 = Plant death. The percentage of severity of injury was calculated according to the Mckinney formula (1923) as follows:

The severity of the infection=[(No. of plants per degree 0×0)+...(No. of plants per degree 5×5)\ Total Plants ×5]=×100.

Design and analysis of variance

The tests were carried out according to the Complete Randomized Design (C.R.D). The differences between the mean were compared with the Least Significant Difference (L.S.D = 0.05). The program Genstat (2001) was used in the statistical analysis of the experiments by Dr. Ahed Abd Ali Hadi.

Results and Discussion

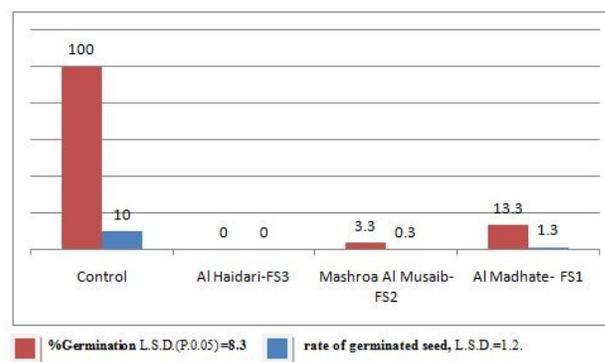
Isolate of *Fusarium solani* from infected plant parts

The results of isolating the fungus from the affected plant parts of the tomato seedlings (roots and proximal stem areas) from the samples collected during the visit to the tomato fields showed the appearance of 3 isolates of the

Fusarium solani fungus. These isolates showed their rapid growth and formation of Microconidia and Macroconidia. This result is consistent with what many researchers found that one of the causes of root rot disease of the main tomato is *F. solani* fungus (Lucas *et al.*, 1985; Agrios, 2005). The results of the isolation showed the appearance of a number of fungi associated with infected roots such as *Aspergillus niger*, *A. flavus*, *Fusarium* sp., *Penicillium* sp. *Macrophomina phasiolina* and *Rhizopus* sp. With lower incidence rates that were diagnosed and studied separately for subsequent studies.

Test the pathogenicity of *Fusarium solani* isolates using tomato seeds

The results of the pathogenicity test (Fig. 1) showed that all the isolates of the fungus had a high pathogenicity, where the number of seeds grown ranged from 0.0-1.3 seeds. The percentage of germination of tomato seeds was between 0-13.3% compared with the control treatment which was 100%, Fs-3 isolate (Al-Haidari district) was superior to others, as it prevented the germination of the seeds completely followed by without significant difference with the Mashroo Al Musaib district. The effect of seed germination is due to the fungal secretion of the enzymes of the pectin and cellulose, which are responsible for rotting in seeds and thus preventing them from germination (Shenashen *et al.*, 2017).



FS= *Fusarium solani*, The number beside the symbol represents the number of isolation.

Fig. 1 : Effect of isolates of *Fusarium solani* in the germination of tomato seeds under laboratory conditions.

Test for the antagonistic potential of *Trichoderma harzianum* and *Bacillus thuringiensis* against *Fusarium solani* fungus in the PDA medium.

The results showed that *T. harzianum* (Th) was effective against the *F. solani* and with a high inhibition rate. The biological agent was measured by a contrasting degree 1 according to the scale of Bell *et al.* (1982). The results agreement with Basco *et al.* (2017) of the efficacy of *T. harzianum* for high tolerance to *Fusarium* pathogenesis under laboratory conditions. The efficiency of *T. harzianum* may be due to several reasons that have made this fungus an anti-biotic agent against many pathogenic fungi of the plant, including fungus causing root rot. These are the direct Mycoparasitism on the mycelium of the fungi by twisting around his strands and analyzing the walls by the enzymes. And the production of antibiotics that negatively affect the growth of pathogenic fungi (Harman, 2004). The possession of *T. harzianum* is highly competitive on the space and the

food is rapidly growing and possessing high inoculum potential (Adekunle *et al.*, 2006).

The results showed that bacteria *B. thuringiensis* had a very low inhibitory effect (Fs-3). this is due to the weakness of isolate or lack of antagonism ability to the trend towards the fungus pathogen and inhibited growth under laboratory conditions as a bacteria specialized in the control of some types of insect pests or this isolation may be very weak.

Effect of the water extract of the leaves of the plant of the *Conocarps* and Rough cocklebur in the growth of the fungus *Fusarium solani* on the PDA medium

The results showed (Table1) that the water extract of the leaves of the *Conocarpus* and the Rough cocklebur (5, 10 and 15%) was highly effective against *F. solani* isolates (Fs3) and significantly superior to the extract of the leaves in 15% which caused complete inhibition of pathogen that was 100%. While the concentration of 5 and 10% reduced the growth of fungus with a growth rate 3.467 and 1.933% respectively, the rate of inhibition was 61.48 and 78.42%. While the water extract of the leaves of the *Conocarpus* plant significantly reduced the growth rate of the fungus ranged between 4.333-6.400 cm and the rate of inhibition ranged from 51.85 to 28.89%. The reason for the efficiency of the leaves of the plant of the *Conocarpus* and the Rough cocklebur Extract may be due to the fact that it contains many chemical compounds (resinous substances and balsam Waxes, essential oils, flavons, elements, organic substances, etc.) against a very large number of microorganisms (Lokendra and Sharma, 1978; Matloob and Alkaif, 2016). The toxicity of these extracts may be due to the containment of the *Conocarpus* on alkaloids, glycosides and sappiness (Putna, 1987). The results are consistent with a study that found that the alcoholic extract of Rough cocklebur, the Rough cocklebur plant has a reaction to a number of microbes, including *Aspergillus flavus* and *Fusarium solani* (Fazli *et al.*, 2012).

Effect of *Trichoderma harzianum* fungus and *Bacillus thuringiensis* and plant extract in the disease incidence and the severity of damping off seedling disease of tomato and some of the criteria of plant growth under field conditions

The results of this experiment (Table 2) showed that the treatments used reduced the negative effects of pathogenic fungus and clearly provided good protection for tomato

plants from infection. The treatment of interference between *T. harzianum* and *B. thuringiensis* and the extract of the Fs-3 protected the seeds and improved their germination percentage to be treated with 88.88% followed by no significant difference between the treatment of *T. harzianum* and the extract of the Rough cocklebur, which reached 85.18% compared with the germination rate of the fungus pathogen, which had a germination rate of 33.33%. The results showed that the addition of the biological agents and the extract of Rough cocklebur, single or integrated, reduced the disease incidence and severity of the *F. solani*, which is the cause of the damping off disease and the root rot of the tomato. The integrated treatment between the *T. harzianum* and the bacteria and Rough cocklebur was superior to treatments and the presence of fungus in reducing the disease incidence and severity of infection to 25.92% and 13.33% respectively and close to the treatment of chemical pesticide Beltanol, which was the disease incidence and severity of infection at 14.81 and 8.14%, respectively. The results showed the effect of *T. harzianum* and the extract of the Rough cocklebur were intertwined with the presence of fungus in reducing the disease incidence and severity of infection to 33.33 and 28.88% respectively. The two factors alone showed a high efficiency in reducing the disease incidence and severity of the fungal infection by *F. solani* causing agent of damping off seedling disease and rot root of the tomato under the conditions of the plastic house. The results showed that the addition of the bio-control agents and the extract of the Rough cocklebur plant were overlapping with each other. It improved the studied plant growth parameters of plant height, wet and dry weight of the plants and superior treatment of *T. harzianum* and *B. thuringiensis* and the extract of the Rough cocklebur with the presence of fungal pathogen, as the length of the plant then 6.667 cm, measured by the treatment of fungus pathogen with a single plant length was 3.233 cm. The coefficients also increased the weight of the wet plant, ranging between 0.533-1.693g and dry weight between 0.137-0.697g, respectively. The fungus *T. harzianum*, because of its parasitic nature, is able to produce a number of enzymes that help in the analysis of the walls of host cells, notably glucanases, Chitinases and Cellulase, which contribute to the analysis of polysaccharides, Chitin and B-glucans, Its hardness and then affect the safety of cellular walls of pathogenic fungi (Ulker *et al.*, 2011). As well as that *T. harzianum* works to inhibit the active of pathogenic fungi.

Table 1 : Effect of the water extract of the leaves of the plant of the *Conocarps* and the Rough cocklebur in the growth of the *Fusarium solani* on the PDA.

Inhibition (%)	Growth rate (cm)	Concentrates (%)	Treatments*
0.0	9.0	0	Fs3
61.48	3.467	5	Fs3 + <i>Conocarpus</i>
78.42	1.933	10	
100.00	0.000	15	
28.89	6.400	5	Fs3 + Rough cocklebur
40.37	5.367	10	
51.85	4.333	15	
3.847	0.346	-	LSD (0.05)

*Each number represents the rate of three replicates, Fs-3 = *Fusarium solani*, isolation 3 (Babylon-AIHaidari)

Table 2 : Efficiency of *Trichoderma harzianum* and *Bacillus thuringiensis* in the in the disease incidence and the severity of damping off seedling disease of tomato caused by *Fusarium solani*.

Plant weight (g)		Length of plant (cm)	Disease severity (%)	Disease incidence (%)	Seed germination (%)	treatments*
Dry	Wet					
0.125	0.480	3.233	77.77	96.29	33.33	Fs-3
0.413	1.367	4.667	43.70	59.25	77.77	Fs-3+Th
0.137	0.533	3.467	75.70	88.88	40.74	Fs-3+Bt
0.437	1.233	4.500	46.66	66.66	74.07	Fs-3+RE
0.487	1.433	5.833	30.36	37.03	85.18	Fs-3+ Th+Bt
0.567	1.583	5.100	28.88	33.33	88.88	Fs-3+ Th+RE
0.583	1.567	6.333	28.88	40.74	88.88	Fs-3+Bt+RE
0.697	1.693	6.667	13.33	25.92	100.00	Fs-3+ Th+Bt+RE
0.537	1.467	5.333	8.14	14.81	100.00	Fs-3+Bel
0.570	1.520	5.667	0.00	0.00	100.00	Control
0.766	1.713	7.133	0.00	0.00	100.00	Th
0.533	1.533	6.133	0.00	0.00	100.00	Bt
0.667	1.633	5.467	0.00	0.00	100.00	RE
0.803	1.733	19.33	0.00	0.00	100.00	Th+Bt
0.833	1.766	7.067	0.00	0.00	100.00	Th+RE
0.667	1.653	7.733	0.00	0.00	100.00	Bt+RE
0.833	1.870	7.900	0.00	0.00	100.00	Th+Bt+RE
0.0140	0.0823	0.634	1.790	6.534	5.334	LSD

*Each number represents one treatment rate with three replicates, Fs-3 = *Fusarium solani*, isolate 3 (Babylon-Haidari), Bt = *B. thuringiensis*, Th= *Trichoderma harzianum*, RE= Rough cocklebur Extract, Bel = Beltanol.

T. harzianum is a fierce competition for this type of rapid growth and quick colonization of the base material, which leads to the elimination of pathogenic fungi (Adekunle *et al.*, 2006; Harman, 2006). The results showed that all the treatments resulted in a significant increase in the growth criteria of the studied peat seedlings and found significant superiority of the integration treatment on all biological control agents in increasing plant length and wet and dry weight which was 7.900 cm, 1.870 g and 0.833 g, respectively. The results showed that *B. thuringiensis* (Bt) did not show a high efficiency in reducing the disease incidence and severity of plant injury as it was 40.74% and 88.88, respectively.

Conclusions

The conclusions of this study that *F. solani* is the main cause of the damping off of seedling and rot root of tomato in the areas from which the samples were collected in Babylon province, where isolation was done. The use of the biocontrol agent *T. harzianum* or *B. thuringiensis* and the Rough cocklebur extract resulted in a reduction in the disease incidence and severity of the disease caused by *F. solani*. Using a combination of *T. harzianum* and *B. thuringiensis* bacteria and Rough cocklebur extract achieved a high efficiency in controlling the rot root disease of tomato and positive results in increasing the growth parameters of the plant under field conditions.

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