

SOYBEAN SEED BORNE FUNGI AND THEIR CONTROL 2- EFFECT OF SOIL AMENDMENTS ON THE INCIDENCE OF FUSARIUM ROOT ROT AND CHLAMYDOSPORES GERMINATION

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Abstract

The addition of mature (high C:N ratio) plant residues, and two immature (low C:N ratio) residues of barley and sorghum to soil, significantly reduced *Fusarium* root rot of soybean. Disease rating was positively correlated with total soil inorganic nitrogen and nitrate. Soil amendment with residues of mature or immature barley and mature oat appreciably increased stem length and fresh root weight of Clark soybean. Mature and immature residues of barley or oat decreased *Fusarium* population in soybean roots or rhizosphere, especially after 80 days from addition of these residues; however, other residues had no effect. Addition of soybean residues to the soil increased the incidence of soybean seed infected by *Fusarium oxysporum*, while soil amendment with barley or oat residues had a reverse effect.

Decomposing mature residues were more inhibitory to chlamydospore germination *in vitro* and in soil than the corresponding immature residues. Failure of chlamydospores to germinate *in vitro* and in soil was related to nutrient deficiency in the soil as well as to the formation of an inhibitory material from the decomposing residues. Fungistasis due to nutrient deficiency, but not toxicant production, was overcome in soil in the presence of nutrients.

To improve physical, chemical and other soil properties for any soil contaminated with *Fusarium* root rot the addition of mature crop residues of high C:N ratio is recommended.

INTRODUCTION

Wilt of soybean [*Glycine max* (L.) Merr.] caused by *Fusarium oxysporum* Schlecht, emend. Snyder & Hans. has been investigated (Leath and Carroll, 1985 and Lee 1986). *F.oxysporum* has been studied as a primary pathogen and as a part of the root rot complex (French and Kennedy, 1963). Although this disease has caused great crop losses in the soybean-growing areas of Upper Egypt for many years, its control is still in its infancy. Chemical control currently is either not available or is too costly. Cultural control which employs a reduction of soil compaction in conjunction with appropriate irrigation has been a successful method (Burke *et al*, 1972; Khalifa, 1993 and Yehia *et al*, 1994). Unfortunately, methods for control which in-

volve plant residue incorporation into the soil has met varied success. In spite of the difficulties inherent in this approach, the stress on the importance of preserving the environment requires continued exploration into the area of biological control for the suppression of soil-borne plant pathogens. For many years, various organic amendments and plant tissues applied to soil were shown to reduce *Fusarium* root rot of beans in the laboratory and greenhouse (Cook and Watson, 1969; Papavizas *et al.*, 1968 and Arafa, 1994).

Decomposing plant residues can significantly affect soil-borne plant pathogens by alteration of pathogen inoculum level. Of major importance in this respect is the carbon: nitrogen (C:N) ratio of the amendments (Adams *et al.* 1968; Papavizas *et al.*, 1968 and Toussoun *et al.*, 1963). Population levels, in turn, are affected by the extent of pathogen chlamydospore germination. In soils amended with residues that contain stimulatory nutrients, chlamydospores germinate and can form replacement chlamydospores so that the numbers increase (Lewis and papavizas, 1977). Some amendments of high C:N ratio, such as cellulose or oat straw, allow only slight, or no chlamydospore germination (Papavizas *et al.*, 1968). The inhibition of chlamydospore germination due to decomposing mature amendments generally has been attributed to soil fungistasis arising from nutrient deficiency (Watson and Ford, 1972). In this respect, Subba Rao (1975) reported that the rate of immobilization of nitrogen depends on the C:N ratio of the organic matter added. The critical balance between mineralization and immobilization may be upset if the C:N ratio is less than 25 when mineralization is likely to exceed immobilization leading to accumulation of ammonium and nitrate forms of nitrogen.

This work provides additional information on the effect of decomposing some plant residues of *Fusarium* root rot, stem length, fresh root weight of soybean and population of the pathogen in the root or soil rhizosphere of soybean and the effect of residues extracts on chlamydospore germination *in vitro* and in soil.

MATERIALS AND METHODS

A. Soil, isolate and residues:

The soil used was sandy loam having a pH of 7.6, containing 31 and 27 ug of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N/g}$ soil, respectively, and the organic matter content was 2.6%. One isolate of *F.oxsporum* was previously isolated from discolored soybean. Plant residues, composed of leaves, stems and roots were air-dried and ground in a mill to pass a 0.84-mm (20 mesh) screen. Total carbon and nitrogen contents were determined as discribed by Jackson (1958). This was performed at the Laboratory of

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Soil, Water and Plant Analysis, Faculty of Agriculture, El-Minia. The residues of each plant consisted of 5 sets of plant tissues; one of each pair being immature (low C:N ratio), and the other being mature (high C:N ratio). Plants included in this study and their respective C:N ratios were : oat (*Avena sativa* L.) 8 and 73; sorghum (*Sorghum vulgare* L.) 10 and 58; corn (*Zea mays* L.) 8 and 70; barley (*Hordeum vulgare* L.) 8 and 76 and soybean [*Glycine max* (L.) Merr.] 10 and 56.

Available $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in soil were determined by steam distillation with magnesium oxide-Devarda alloy method (Bremner and Keeney, 1965). The equivalent of 10 g dry weight of each treatment replicate soils were analyzed prior to planting.

B. Effect of plant residues on soybean root-rot, stem length, fresh weight and population of pathogen in both soybean roots and rhizosphere.

Soil was infested with *F.oxysporum* by adding microconidia suspension (200 ml.) to the soil and keeping it moist for 5 weeks in order to convert conidia to chlamyospores (Nash *et al.*, 1961). Number of propagules of the pathogen in the soil was determined by the dilution method and plating onto Nash and Snyder medium (1965) amended with chloramphenicol (300 ug/ml.). Plant residues of the previously mentioned crops were added and mixed thoroughly with the infested soil at a rate of 1.0% (w/w) and moistened to 50-60% of their moisture holding capacity. After 3 weeks, infested and treated soil was placed in 6.5 cm square plastic pots and planted with 10 Clark soybean seeds per pot. Four replicates were used for each treatment. Soil containing *F.oxysporum* only served as control. Disease rating, stem length and fresh root weight were recorded after 7 weeks from planting. Populations of *F.oxysporum* were determined after 50 and 80 days following the addition of plant residues both in the roots and rhizosphere of Clark soybean.

At the time of harvest, 25 seeds of each treatment were shaken in 1.25% Na OCl for 3-4 minutes, rinsed in sterile distilled water, and cultured on Difco potato-dextrose agar (PDA). Incubation was made at 20-22°C, usually for 7 to 10 days (Ferrant and Carroll, 1981), when counts of infected seeds were made and growing fungi were identified.

C. Effect of decomposing barley and corn residues on chlamyospore germination.

Chlamyospore preparations were tested for germinability by the method of

Adams *et al.*, (1968), which is a modification of Jackson's agar disk technique (Jackson, 1958). 20-g aliquot of soil amended with (mature or immature residue of each of barley and corn or nonamended soil) was mixed with 10 ml. of warm (50°C) 0.5% agar with or without 0.1% potato dextrose broth (PDB) powder. The soil agar mixture was placed in petri dishes and after it had set, three disks (1x10 mm) of 2% agar, with or without 0.1% PDB powder, were placed on the soil-agar surface, kept at 5°C for 48 hr, then a drop of diluted chlamyospore suspension (50,000/ml.) was placed on each disk. After incubation at 26°C for 18 hr, disks were transferred to microscope slides, stained with lactofuchsin, and examined.

D. Effect of residue extracts of barley and corn on chlamyospore germination.

Extracts of residues decomposing in soil were prepared by extracting 1 kg portions of amended soil (2%) with chloroform: methanol (1:1 v/v) for 24 hr. The extracts were concentrated under vacuum to a brown, viscid material. Non-amended soil, soil amended with immature barley and corn, and with mature barley and corn yielded 140, 280, 310, 450 and 420 mg of extract/kg of soil respectively. Extracts were homogenized with water in a blender so that an amount equivalent to that found in 2% nonextracted residue was added to the soil containing chlamyospores. Glucose and NH_4Cl were added to the soil to stimulate germination. Germination of *F.oxysporum* chlamyospores was determined by the propagule assay method 16 hr after the extract was added to the soil (Lewis and Papavizas, 1977).

RESULTS

A. Effect of decomposing plant residues on :

A. 1. Soybean root rot and soil inorganic N content:

All mature residues reduced disease below those recorded in the nonamended control soil (Table. 1), whereas only two immature residues (barley and oat) were effective in this respect. Barley and oat residues were the most effective mature amendments to reduce the disease rating in Clark soybean plants. Similar results were obtained when the experiment was repeated.

At the time of planting, soil inorganic N content varied between 34.5 ug/g in mature oat-amended soil and 93.3 ug/g in immature corn-amended soil. Soil $\text{NH}_4\text{-N}$ contents were approximately the same in all amended soils, with the exception of

immature corn-amended soil which contained a significantly greater amount than soils amended with other residues. In contrast, soil $\text{NO}_3\text{-N}$ varied considerably among treatments. There was generally less of this form of N in soils amended with mature than immature residues.

Two of the five plant residues (barley and oat) which reduced disease in a significantly lower content of $\text{NO}_3\text{-N}$ in the soil than the other three (Table 1). Disease rating was positively correlated with total inorganic nitrogen and $\text{NO}_3\text{-N}$ content. The correlation between disease rating in Clark soybean and $\text{NH}_4\text{-N}$ content was not significant.

Table 1. Effect of decomposing immature and mature plant residues on root rot of Clark soybean caused by *F.oxysporum* and the total inorganic nitrogen content of soil at the time of planting.

Plant residues (a)		Disease rating after 7 weeks for planting (b)	Inorganic N (ug/g)		
			$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	Total
Barley	immature	2.0	23.4	26.4	49.8
	mature	1.4	24.6	14.1	38.7
Oat	immature	3.6	22.5	29.7	52.2
	mature	2.0	21.1	13.4	34.5
Sorghum	immature	5.2	21.3	56.0	77.3
	mature	2.8	25.6	19.0	44.6
Corn	immature	5.4	35.1	58.8	93.9
	mature	4.5	20.0	22.2	42.2
Soybean	immature	7.0	21.9	45.2	67.1
	mature	4.6	23.0	20.2	43.2
No residue (control)		7.4	24.2	40.2	64.4
L.S.D.	immature (A)	2.01	4.21	3.23	2.14
	5% mature(B)	1.70	3.06	2.17	3.21
	A x B	0.92	5.12	4.02	6.62

a. Added to soil at rate of 1% (w/w)

b. On a 1-10 scale where 1 = healthy plant, 10 = dead plant.

A.2. Stem length and fresh root weight of Clark soybean:

Data in table (2) indicate that soil amendment with residues of mature or immature barley plants and mature oat significantly increased fresh root weight and stem length of Clark soybean plants, while residues of other plants were of no ef-

fect. Highest increase was experienced with mature barley-amended soil.

At the time of harvest, percentage of infected seeds were determined for each treatment. All mature and immature residues decreased the percentage of seeds, infected with *F.oxysporum*, when compared with nonamended control soil. Adding barley residues both mature or immature had the most effect in decreasing the percentage of seeds infected with *F.oxysporum* [4.4 and 6.4 respas compared to the control (32.4), while the residues of both mature and immature soybean had the least effect in decreasing this percentage [22 and 30.2 resp. as compared with control (32.4)]. Other residues had intermediate effects.

Table 2. Effect of decomposing immature and mature plant residues in soil infested with *F.oxysporum* on stem length, fresh root weight and percentage of infected seeds of Clark soybean var.

Infested soil amended with plant residues		Stem length (cm.) (a)	Fresh root weight (g)	%, infected seed harvested
Barley	immature	17.2	2.1	6.4
	mature	20.4	2.8	4.4
Oat	immature	15.5	1.1	8.2
	mature	16.4	1.9	9.0
Sorghum	immature	12.3	0.8	16.8
	mature	13.7	0.9	18.6
Corn	immature	14.6	1.2	14.5
	mature	15.3	1.4	12.1
Soybean	immature	10.4	1.6	30.2
	mature	9.7	1.2	22.0
Noresidue (control) (c)		12.1	1.0	32.4
L.S.D.	immature (A)	1.94	0.91	4.21
5%	mature(B)	2.12	0.70	3.61
	A x B	3.04	1.01	6.24

a. Mean of four replications of five plant each.

b. Mean of four replication of three plant each.

c. No plant residues was amended with soil infestation.

A.3. Population of *F.oxysporum* in soybean roots and soybean rhizosphere.

Data in table (3) show that soil amended with residues of immature and mature barley and oat significantly decreased *F.oxysporum* population recorded in both

soybean roots and rhizosphere after 50 and 80 days from addition of residues, whereas soil amended with soybean residues increased fungal population at the same dates. Other treatments were of no effect, except in immature and mature corn-amended soil where a reduction of fungal population in the rhizosphere was recorded after 50 days only.

A significantly larger *F.oxysporum* population was isolated after 80 days either from roots or the rhizosphere of Clark soybean than those isolated after 50 days from addition of residues. Mature barley residues caused the highest decrease in fungal population in Clark roots and rhizosphere at the two periods; however, mature or immature residues of soybean resulted in the opposite.

Table 3. Effect of decomposing immature and mature plant residues on isolation of *F.oxysporum* from Clark soybean roots and rhizosphere soil.

plant residues (amended with soil infestation a)		Population of <i>F.oxysporum</i> from Clark soybean after addition of plant residues			
		Roots (b)		Rhizosphere soil of plants (c)	
		after 50 days	after 80 days	after 50 days	after 80 days
Barley	immature	21.0	32.4	62.4	88.4
	mature	18.4	25.6	55.0	80.0
Oat	immature	26.0	37.2	69.0	90.1
	mature	23.0	30.0	60.3	84.2
Sorghum	immature	36.0	46.5	76.9	98.5
	mature	34.9	44.2	74.5	97.2
Corn	immature	38.4	43.8	70.2	99.3
	mature	37.2	41.6	69.8	101.7
Soybean	immature	40.0	66.4	95.4	122.0
	mature	46.2	70.2	92.2	119.4
No residue (control)		38.0	48.5	79.3	103.4
L.S.D. 5%	immature (A)	2.95	3.92	5.07	5.92
	mature(B)	5.23	4.82	3.62	6.07
	A x B	7.01	5.23	6.34	8.14

- a. Initial inoculum density of *F.oxysporum* (2×10^3 propagules/g soil).
b. Mean frequency of isolation from 100 root section averaged over four replicates and two sampling dates.
c. Mean number of propagules (1: 100 dilution) averaged over four replicates and two sampling dates.

B. Effect of decomposing barley and corn residues on chlamydo-spore germination.

Barley and corn residues were selected for further study taking into consideration that mature barley was the most effective amendment in disease reduction, while the mature residues of corn was the least effective.

With the agar-disk assay method, both mature residues significantly reduced germination of *F.oxysporum* chlamydo-spore on agar over a 15-wk period (Fig.1). However, mature barley amendment was more superior in its effect. Barley immature residues reduced germination by approximately 22.5%, while reduction was 16.0% in case of immature corn. Reduction in germination occurred in the presence of nutrients (PDB powder) supplied to the assay media (Fig 1-A). When these nutrients were not supplied, chlamydo-spore germination was high at the time the amendments were added, but not during the remainder of the 15-wk test period (Fig 1-B).

C. Effect of residue extracts on chlamydo-spore germination.

Extracts from decomposing mature barley and corn residues inhibited chlamydo-spore germination in soil even in the presence of nutrients (table 4). Significantly greater inhibition occurred with mature barley than with mature corn. For decomposing immature residues, corn and barley had a little effect on germination of indicated *F.oxysporum*.

Table 4. Effect of chloroform methanol extracts of decomposing barley and corn on *F.oxysporum* chlamydo-spore germination in soil as determined by the prop-agules assay method. (a).

plant residue extract (b)	Nutrients (c)	Germination (%) of chlamydo-spores
None d)	+	88
Control	+	86
Barley (C:N 8)	+	66
Barley (C:N 76)	+	20
Corn (C:N 8)	+	79
Corn (C:N 70)	+	50
LSD 5%		12.32

a. Residues extracted 3 wk after addition to soil.

b. Added at a rate equivalent to that found in 2% soil amendment.

c. Glucose and NH₄Cl each added at 140 ug/g of soil.

d. "None" indicates no extract from nonamended or amended soil was added. "Control" indicates extract from nonamended soil was added.

DISCUSSION

The importance of high C:N ratio materials such as mature barley, corn and sorghum residues in the suppression of *Fusarium* root-rot of bean was shown experimentally as early as 1959 (Cook and Watson, 1969). However, relatively little work has been done to unravel possible mechanisms of control by these amendments.

From the obtained results in table (1) it may be stated that low root rot severity resulted from a decrease in population of the fungus in both soybean roots and rhizosphere is brought about by high C:N ratio residues. These results are also positively correlated with low total inorganic nitrogen and low $\text{NO}_3\text{-N}$. These actions can be attributed to nitrogen immobilization by soil microbial competitors to *F. oxysporum*, which was thought to be the mechanism of action of high C:N ratio residues (Lewis and Papavzas, 1977; Papavzas *et al.*, 1968). Subba Rao (1975) reported that the rate of immobilization of nitrogen depends on C:N ratio of added organic matter.

Disease rating was shown to be positively correlated with $\text{NO}_3\text{-N}$ content of soil. This result is in agreement with the finding of Huber and Watson (1974) and Paulitz and Burke (1987). However, some mature residues (oat and barley) were more effective than others (sorghum, corn and soybean). Soil N content alone could not account for disease suppression or increase (Lewis and papavizas, 1977). In this study, decomposing mature barley and oat which have high C:N ratios (76 and 73 resp.) resulted in nearly the same amount of inorganic N (34.4 and 38 $\mu\text{g/g}$), but each of the two materials had a different disease rating (1.4 and 2 resp). Burke (1969) suggested that the N status of soil may not be the major determinant in amendment effectiveness for suppression of *Fusarium* root rot.

As show in table (2) soil amendment with residues of mature and immature barley plants and mature oat significantly increased fresh root weight and stem length. Subba Rao (1975) attributed the effect of humic substanes as a final products of organic matter decomposition on improving root growth and reported that the growth stimulatory activity of humus complexes on plant root may be attributed to the increase in cytochrome oxidase activity in the root system. Similarly, humic acids are known to increase the activity of glutamic acid transaminase and phosphorylase enzymes and also the synthesis of deoxyribose and ribose nucleic acids.

Since N content of soil could not explain why some amendments were more effective than others, attempts were made to determine whether amendment decom-

position in soil had any adverse or beneficial effects on chlamyospore germination. With the agar-disk method, the lack of germination as a result of amendment decomposition was due to fungistasis arising from nutrient deficiency (Fig 1-B). Both barley and corn residues, regardless of maturity, resulted in a fungistatic soil. This agrees with earlier observations (Adams *et al.*, 1968; Bristow and Lockwood, 1979 and Papavizas *et al.*, 1968), who reported that decomposing cellulose and other organic materials prevented chlamyospore germination in soil.

As nutrients were added as PDB powder to both agar disks and to the soil agar mixture after 15-wk of amendment decomposition, chlamyospore germination ranged between 67.5% and 73% with immature residues of barley and corn respectively, compared to 91.5% in the control; however, this decrease was most pronounced with mature residues of these crops (Fig 1-A). Lewis and Papavizas (1977) found that adequate nutrients for germination were present in agar disk over mature rye-and corn amended soil. Despite this, germination was poor in these treatments. This poor germination may be due to some other reasons.

The possibility of formation of a toxic principle was investigated. Previous evidence for production of an extractable toxicant against soil borne plant pathogens has been minimal (Watson and Ford, 1972). Evidence based on our data with agar-disk method, suggests that a factor toxic to chlamyospore germination was associated with mature amendment decomposition in soil. In the present study, a direct evidence for a toxicant was made by extracting a crude preparation of the toxic material from decomposing mature barley and corn residues and demonstrating its inhibitory effect on chlamyospore germination with the propagule assay method (Table 4). Other reports attributed the inhibition of chlamyospore germination by decomposing mature amendments to soil fungistasis arising from nutrient deficiency (Griffin *et al.*, 1975; Lewis and Papavizas, 1977).

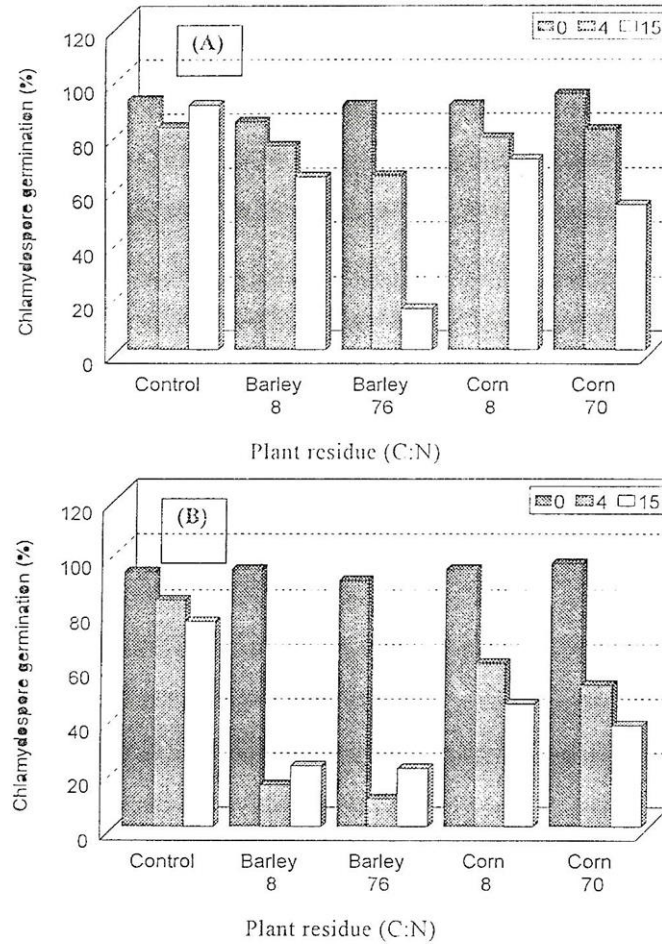


Fig.1. (A-B). Effect of decomposing barley and corn residues on chlamydespore germination of *F.oxysporum* determined with agar-disk method. A) Nutrients as potato dextrose broth powder (0.1%) added to disks and soil agar mixture. B) No nutrients added. Assays performed, 0, 4 and 15 wk after addition of residues (1%) to soil. Germination represents percentage of that on agar plates alone with nutrients (75%) and without (80%).

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دراسات على الفطريات المصاحبة لبدور فول الصويا وطرق مقاومتها
 ٢. تأثير إضافة بقايا بعض المحاصيل إلى التربة على إنبات
 الجراثيم الكلاميديه للفطر فيوزاريوم اكسسبوريوم
 وكذلك على عفن جذور فول الصويا

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إضافة بقايا بعض المحاصيل الناضجة والعالية فى نسبة الكربون للنيتروجين وكذلك بقايا محصولى الشعير والذره الرفعية الغير ناضجة والمنخفضة فى نسبة الكربون للنيتروجين الى التربة أدت إلى حدوث خفض معنوى للإصابة بمرض عفن الجذور الفيوزاريومى فى فول الصويا، كما وجد إرتباط بين نسبة الإصابة بالمرض وكلامن النيتروجين الكلى الغير عضوى وصورة النترات فى التربة.

إضافة البقايا الناضجة والغير ناضجة لحصول الشعير وأيضا البقايا الناضجة فقط لحصول الشوفان أدت إلى زيادة فى كل من طول الساق ووزن الجذر الطازج لصنف فول الصويا كلارك.

إضافة بقايا محصول الشعير والشوفان سواء الناضجة أو الغير ناضجة أدت الى نقص فى أعداد جراثيم الفطر فى جذور فول الصويا أو بمنطقة الريزوسفير وبالأخص بعد ٨. يوم من إضافة هذه البقايا، بينما إضافة المحاصيل الأخرى لم يكن لها أى تأثير.

تحلل بقايا المحاصيل الناضجة كانت أكثر تأثيرا فى تثبيط إنبات الجراثيم الكلاميديه سواء فى المعمل أو فى التربة عند المقارنة بتأثير المحاصيل الغير ناضجة.

لتحسين الصفات الكيماوية والطبيعية وبعض الصفات الأخرى للتربة الملوثة بفطر الفيوزاريوم اكسسبوريوم يوصى بتأضافة بقايا المحاصيل الناضجة ذات النسبة العالية من الكربون للنيتروجين.