

## Non-chemical Control of Potato Early Blight Caused by *Alternaria alternata*

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### ABSTRACT

Early blight of potato caused by *Alternaria alternata* (Fries) Keissler is one of the factors that affect potato production. Using chemical control to reduce disease severity represent another risk for agriculture. Biological control, using microorganisms, as well as plant extract and compost are safe measures that give reliable control. Applying either measure of control reduced disease incidence to less than 2% during two successive seasons. Microbial spray of diseased potato reduced the disease to various extents. *Trichoderma* sp. was the most effective among all tested microorganisms, followed by *Penicillium* sp. On the other hand, alcoholic extract of three plants was more effective than their water extract. However, water extract showed highly significant reduction of disease incidence. *Polygonum* gave the highest reduction of disease incidence in both cases. Also water extract of compost greatly suppressed early blight when applied as spray to potato leaves. Duration of extraction may affect the ability of compost extract to suppress disease. All results were comparable to that of fungicides.

**Key words:** *Trichoderma*, compost, plant extract, potato blight, non chemical control.

### INTRODUCTION

Potato plant (*Solanum tuberosum* L.) is one of the most popular carbohydrate crops all over the world. Fungal diseases are among the constraints that affect economical production of potatoes. Early blight is one of these important diseases. It has been known for a long time to be caused by *Alternaria solani* (Ellis et Martin) Sorauer. Nevertheless, *Alternaria alternata* flashed out, recently, as a new causative agent of this serious disease (Boiteux and Reifschneider, 1994; Abdul Wahid *et al.*, 2005).

Conventionally the disease is controlled by chemical fungicides. However, owing to the growing awareness of publics about the risk of chemicals as well as the world transfer towards the organic agriculture systems, eco-safe and hazardous free measures for diseases control are urgently needed. This could be achieved through the implementation of non-chemical strategies. An environmentally sound strategy that has not been explored for early blight management is the compost tea. Compost is used traditionally to promote plant growth, but its effect on soil borne fungal pathogen cannot be neglected. Moreover, application of compost tea to foliage has been documented for some other pathosystems (Weltzien, 1991; Scheuerell and Mahaffer, 2002; Ryan *et al.*, 2005). Another strategy that has been employed to improve crop production and suppress fungal diseases is plant extract. Many wild and cultivated plants have been found to cause marked suppression of plant pathogenic fungi including *A. solani* (Shaudat and Siddiqui, 2002; Al-Mughrabi, 2003). The third strategy relies upon the use of natural enemies, i.e. biological control measure. Successful biological control depends on the use of locally resident antagonistic microorganisms. They should have the ability to suppress the disease progress through

implementation of several mechanisms, such as competition, antibiosis, parasitism, and/or induction of host self defense mechanism. Several fungi are exploited to control many plant diseases. *Trichoderma* species are well known as biocontrol agents of many fungal pathosystems through exertion of different mechanisms (Kommedahl and Windels, 1978; Pascual *et al.*, 2000; Monte, 2001; Howel, 2003).

This investigation tends to explore the potential of some non chemical strategies to suppress early blight disease of potato caused by *A. alternata*. Also, it aims to evaluate the efficacy of these measures under open field conditions.

### MATERIALS AND METHODS

#### Compost tea: preparation and application

Commercial compost, kindly provided by Agriculture Research Center, Station of Ismailia, Soil and Water Department, was used to make compost tea (CT). The compost was added to sterile distilled water (SDW) at ratio of 1: 4 (w/v), and incubated for either 7 or 14 days at ambient temperature. The resulted tea was filtered through cheesecloth then followed by Whatmann No. 1 filter paper to get rid of any plant particles and obtain clarified extract. The resultant CT was applied as spray onto potato foliage leaves.

#### Plant collection, extraction, and application

Three plant species were collected from and around the commercial potato fields at El-Behairah Governorate, where field assessment was carried out (data are not included; Abdul Wahid *et al.*, 2005). The plants were identified as *Polygonum* sp., *Clerodendrum inerme* (L.) Gaertn, and *Cupressus sempervirens* L. The identification was carried out at the herbarium of

Suez Canal University, Faculty of Science. Plants were dried at shaded well aerated place for about 5–7 days. Plants were then extracted either by soaking in SDW over night or by shaking in 70% ethanol, in a ratio of 1:5 (w/v). The extract was then filtered through Whatmann No. 1 and sterilized by Millipore filter (0.2 µm).

The effect of the extract on the radial growth and sporulation of *A. alternata* was tested on potato dextrose agar (PDA) plates supplemented with 3 ml of either plant extract, viz. water or alcoholic. Pathogen plugs (5 mm diameter) from edges of 10 days old culture were placed centrally onto the plates and incubated at 25 °C in the dark.

Meanwhile, for testing the efficiency of the extracts against early blight, the extract was sprayed onto infected potato leaves in the field. Ethanol (70%) or SDW alone were used as control.

#### Isolation of antagonistic fungi

Three methods were adopted for isolation of antagonistic fungi. The first of all was the dilution plate method using soil samples collected from potato growing field at El-Behairah Governorate. Samples were taken at 10 cm depth after removing the top surface layer of the soil (about 5 cm). Crumbs were crushed and soils were sieved to get rid of any plant debris. Aliquots of dilution were dispensed onto PDA supplemented with 500 mg l<sup>-1</sup> chloramphenicol and rose Bengal. The second method was the leaf printing technique. Healthy potato leaves were pressed against PDA plates. The third method was baiting technique using mat of *A. alternata* as bait. *A. alternata* was grown on liquid potato dextrose broth for about 15 days. Excess medium was discarded and the fungal mat was then washed several times with SDW under aseptic conditions. Particles of the previously collected sieved soil were scattered over the mat in 500 ml Erlenmeyer flasks with 10 ml SDW to keep the humid atmosphere necessary for fungal spore germination. Plates and flasks were incubated at 25 °C in the dark. Developed fungal growth was purified, identified, and subjected to antagonistic screen in dual culture, then tested in a field trial.

#### Dual culture

This test was performed to evaluate the potential of isolated fungi to inhibit *A. alternata* growth and to clarify the probable mechanism employed by the candidate used in field experiment. Each PDA plate was inoculated with mycelial plug (5 mm diameter) from the edge of actively growing *A. alternata*. After a period of time, depending on the rate of growth of the pathogen and the candidate, a mycelial plug of either candidate was placed 4 cm apart from the pathogen's plug. Plates were incubated at 25 °C in the dark for 6 days after candidate inoculation. The percentage of growth

inhibition was calculated in relation to *A. alternata* grown in single culture or dual culture with a blank PDA plug. This experiment was conducted twice with three replicates for each fungal candidate. It is worthy to mention that the plates were carefully watched daily to recognize the presence or absence of mycoparasitism as well. Promising candidates were tested in a field trial.

#### Field experiment

Three non-chemical control means were evaluated for their capability to ameliorate the effect of *A. alternata* under open field condition. These were compost tea (7 day and 14 day), 3 plant extracts (water or alcoholic), and 5 antagonistic fungal candidates. Potato leaves (45 days old) were sprayed to runoff with either proposed treatments. After dryness, all plants were challenged with *A. alternata* spore suspension (1x10<sup>5</sup> ml<sup>-1</sup>), except for those who were treated with compost tea where they were challenged 7 days later. All plants were covered with plastic bags overnight to insure infection. Unchallenged plants (blank = natural infestation) and those treated with SDW, alcohol (70% ethanol) or fungicide (Ridomil-Mancozeb, 2.5 g.l<sup>-1</sup>) were served as control. After 14 days of challenge plants were rated for percentage of disease incidence (DI%). The experiment was designed as randomized complete block with five replicas.

#### Inoculum preparation

For field experiment, antagonistic fungi as well as *A. alternata* were cultured on PDA plates and incubated for 10 days at 25 °C in the dark. Plates were then flooded with SDW containing 0.02% Tween 20 and conidia were scraped off using horse-tail hair paintbrush. Spore suspension was adjusted to 1x10<sup>5</sup> using hemacytometer.

#### Calculations and statistical analysis

The percentage of growth inhibition (GI%) and suppression of sporulation (SS%) were calculated according the following equation:

$$GI\% \text{ or } SS\% = \frac{A - B}{A} \times 100$$

Whereas **A** is the growth or sporulation of *A. alternata* alone, and **B** is the growth or sporulation of *A. alternata* in plates containing plant extract or in dual culture.

Data were subjected to ANOVA test and means were separated by the least significant differences (L.S.D.). Data were transformed to arcsine form before performing the analysis. SAS (1988) program was used for data analysis.

### RESULTS

#### Effect of compost on disease incidence

When potato plant was sprayed with compost extract, different degrees of incidence were noticed. In general, the extract suppressed disease at a ratio comparable to that of conventionally used fungicide "Ridomil-

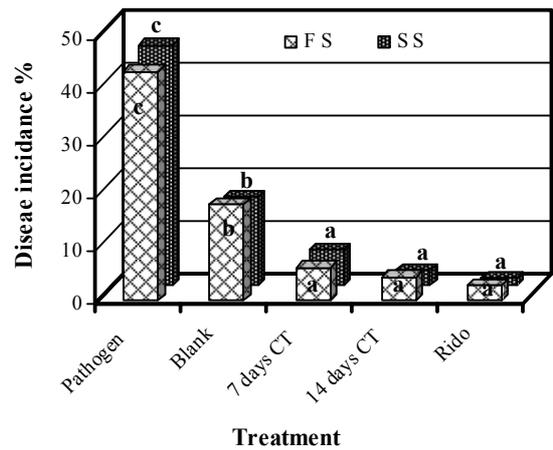
mancozeb”. The 7 days extract reduced disease incidence to 6.12% at the first season. This result was approximately reached during the second season (6.8% DI). The 14 days extract was more efficient than the 7 days extract during both seasons. It was more effective during the second season than the first one (Fig. 1).

**Effect of plant extract on pathogen growth, sporulation, and disease incidence**

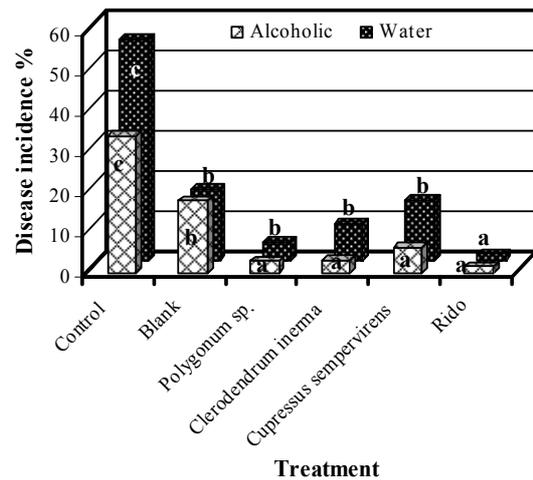
Two plant extracts were used, alcoholic extract and water extract. Both affect pathogen radial growth, sporulation as well as ability to cause disease. Water plant extracts hindered radial growth of *Alternaria alternata* to about 16.5% of the normal growth. The percentage of growth inhibition (GI) varied with the used plant. *Polygonum* sp. was the most inhibitory among the tested plants. It showed 83.52% GI. This was followed by *Clerodendrum inerma* (79.3%). Alcoholic plant extract showed the same trend, but it was more effective than water extract. It almost showed complete suppression of *A. alternata* growth. The growth was shacked to about 2.7% of the normal growth. Alcoholic extract of *Polygonum* sp. gave 97.3% inhibition. On the other hand, alcoholic extract of *Cupressus sempervirens* was not significantly effective although the water extract was significant inhibitor (Table 1).

Pathogen’s sporulation was drastically reduced by both water and alcoholic extract of the three plants. Amount of spores was measured after 14 days of inoculation. *Polygonum* showed the highest activity in suppressing sporulation. It allowed only the production of about 2% of normal sporulation either by water or alcoholic extract. It was followed by *C. inerma* which gave approximately the same effect as *Polygonum*. Percentage of inhibition ranged between 98.05 to 52.86 (Table 1).

When diseased potato plants were sprayed with either water or alcoholic extract they showed different degrees of disease incidence (DI). Extracts, (water or alcoholic), significantly reduced DI, with *Polygonum* sp. being the most potent inhibitory plant. In all cases alcoholic extract was more effective than water extract. It was comparable to that of the fungicide Ridomil-mancozeb, which is routinely used to control early blight (Fig. 2).



**Figure (1):** Effect of compost extract on the percentage of early blight incidence during two successive seasons. Means with the same letters are not significantly different at *p* 0.05 (within the same season). L.S.D. for first season = 7.893; L.S.D. for second season = 5.75. **Rido:** fungicide Ridomil-Mancozeb, **FS:** first season, **SS:** second season.



**Figure (2):** Effect of water and alcoholic extract (70% ethanol) of three plant species on the disease incidence of early blight caused by *A. alternata*. Means with the same letters are not significantly different at *p* 0.05 (within the same extract). L.S.D. for alcoholic extract = 7.14; L.S.D. for water extract = 12.78.

**Table (1):** Effect of water and alcoholic extract (70% ethanol) of three plant species on the pathogen’s radial growth and sporulation.

Treatment	Water extract				Alcoholic extract			
	Diam	% GI	Spor	% SS	Diam.	% GI	Sporu*	% SS
<i>Polygonum</i> sp.	0.9 a	83.5	0.5 a	98.1	0.1 a	97.3	0.3 a	97.9
<i>Clerodendrum inerma</i>	1.13 a	79.3	0.7 a	97.2	0.3 a	91.8	0.3 a	97.9
<i>Cupressus sempervirens</i>	4.03 b	26	7.3 b	71.5	3.34 b	9.7	6.6 b	52.9
Control	5.46 c	---	25.6	---	3.7 b	---	14 c	---
LSD <i>P</i> 0.05	0.766	---	7.845	---	0.769	---	4.92	---

\*: Numbers multiply by 10<sup>5</sup>, Means, within the same column, with the same letters are not significantly different at *P* = 0.05; **Diam:** colony diameter, **GI:** growth inhibition, **Sporu:** sporulation, **SS:** Sporulation suppression.

**Table (2):** Effect of antagonistic fungi on the pathogen's radial growth after 6 days of inoculation of the candidate fungi.

Treatment	Colony diameter (cm)	Growth inhibition %
<i>Trichoderma harzianum</i>	2.03 a	60.96
<i>Trichoderma viride</i>	2.26 a	56.54
<i>Penicillium oxalicum</i>	3.1 a b	40.38
<i>Aspergillus ustus</i>	3.3 b	36.54
<i>Trichothecium roseum</i>	3.4 b	34.62
<i>Alternaria alternata</i>	5.2 c	-----
L.S.D.	0.94	-----

Means with the same letters are not significantly different at  $p$  0.05

**Effect of antagonistic fungi on pathogen's radial growth and disease incidence**

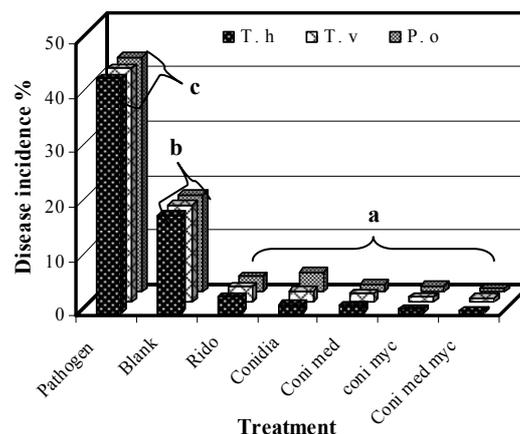
The five used fungal isolates significantly suppressed radial growth of *A. alternata*. *Trichoderma harzianum* Rifai was the most suppressive among them all (60.96% GI) followed by *T. viride* Persoon: Fries (56.54% GI). The lowest percentage of growth suppression was given by *Trichothecium roseum* (Persoon) Link ex S.F. Gray (34.62% GI). It is worthy to mention that in addition to suppression of pathogenic mycelial growth, three of the used fungi were able to parasitize *A. alternata* colony. These were *T. harzianum*, *T. viride*, and *Trichothecium roseum*. They overgrew the pathogen and sporulated on its colony. Applying either one of these antagonistic fungi as a spray to diseased potato plants reduced significantly the disease incidence. *T. harzianum* was the most potent one followed by *T. viride* and *Penicillium oxalicum* Currie et Thom (Fig. 3).

*Aspergillus ustus* (Bainier) Thom et Church and *Trichothecium roseum* were less potent, however, they showed significant reduction of disease incidence comparing to potato plant treated with the pathogen only (Fig. 4). All selected fungi were applied in four preparations: (a) conidial suspension, (b) conidial suspension plus growth medium, (c) conidial suspension plus mycelium, and (d) conidial suspension plus growth medium plus mycelium. The last preparation gave the greatest effect among all other preparations of the five antagonistic fungi. One of the most important results to be mentioned is that some preparations showed priority over conventionally used fungicide.

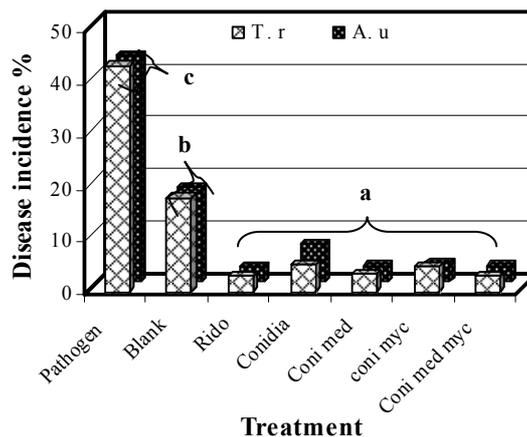
**Discussion**

Non-chemical control represents a promising measure to safe environment and human health from being altered by chemical pesticides. Many strategies have been described depending on the adoption of natural means. Compost is one of the newly developed ways to control plant pests and pathogens. Results of this investigation demonstrated that spraying potato plants with either 7days or 14days compost tea significantly reduced disease incidence and improved potato growth. Application of compost tea reduced DI% to less than 3%. The data is comparable to that of the fungicide "Ridomil-Mancozeb" traditionally used to control

potato blight. Increasing duration of extraction increases the efficiency of the produced tea. Usage of compost to control plant diseases was reported by several workers with other pathosystems (Elad and Shtienberg, 1994). The potential effect of the compost tea could be attributed to the micro biota found in the compost as well as to some chemical compounds synthesized during composting or naturally pre-existed as one of the plant components (Cronin *et al.*, 1996; Sances and Elaine, 1997; Zhang *et al.*, 1998). This potential is greatly affected by nature and quality of compost (Hoitink *et al.*, 1997).



**Figure (3):** Effect of *Trichoderma harzianum*, *Trichoderma viride*, and *Penicillium oxalicum* on the disease incidence of early blight caused by *A. alternata*. Means with the same letters are not significantly different at  $p$  0.05 (within the same isolate). L.S.D. for T. h = 6.3; L.S.D. for T. v = 6.25; L.S.D. for P. o = 6.42. **Rido:** fungicide Ridomil-Mancozeb, **Coni:** conidia, **med:** medium, **myc:** mycelium, **T. h:** *Trichoderma harzianum*, **T. v:** *Trichoderma viride*, and **P. o:** *Penicillium oxalicum*.



**Figure (4):** Effect of *Aspergillus ustus*, and *Trichothecium roseum* on the disease incidence of early blight caused by *A. alternata*. Means with the same letters are not significantly different at  $p$  0.05 (within the same isolate). L.S.D. for T. r = 6.4; L.S.D. for A. u = 6.37. **Rido:** fungicide Ridomil-Mancozeb, **Coni:** conidia, **med:** medium, **myc:** mycelium, **T. r:** *Trichothecium roseum*, and **A. u:** *Aspergillus ustus*.

Several plants contain bioactive compounds that were proved to be active against human fungal pathogens

(Sumner, 2000; Hanson, 2005). The long history of using plant as a natural medication in the folk medicine triggered the conception of exploring the plant extract in ameliorating the pathogenic impact on plants. During this investigation, both water and ethanol extract of *Polygonum* sp., *Clerodendroum inerma*, and *Cupressus sempervirens* showed antifungal activity against the pathogen *A. alternata*. They reduced significantly the growth and sporulation of *A. alternata* on PDA cultures. *Polygonum* and *Clerodendroum* genera were used before against some other pathosystems (Neeta and Sharma, 1992; Sas *et al.*, 1996). When the extract of these three plants was applied to the diseased potato leaves, it reduced DI to a remarkable degree. It is worthy to mention that the alcoholic extract was highly potent than water extract. There was no significant difference between the alcoholic extract (70% ethanol) of the used plants and the conventionally used fungicide "Ridomil-Mancozeb". Difference between the activity of alcoholic extract and water extract could be due to various solubility products of each solvent as well as to polarity of the resulted compounds. However, the water extract still recommended as an alternative to be employed by farmers and growers, for being easy to apply and cheap. The effect of plant extract could be attributed either to the production of antifungal compounds or potent defense elicitors that enhance plant self defense mechanisms (Fofana *et al.*, 2002). It should be noticed that activity of any given plant extract depends on several factors: (a) age of plant at time of harvesting, (b) the used part of the plant, and (c) the method of extraction (Qasem and Abu-Blan, 1996).

Several fungi have been reported as successful powerful biocontrol agents for many diseases (Larena *et al.*, 2002; Spotts *et al.*, 2002). Through preliminary screens, five potent biocontrol candidates were recognized and identified out of about 75 taxa isolated from different sources using various techniques. They showed *in vitro* antagonistic activity against *A. alternata*. The strongest effect was given by *Trichoderma* species (*T. harzianum* and *T. viride*) followed by *Penicillium oxalicum*, which could be considered of a moderate effect. The other two candidates, viz. *Aspergillus ustus* and *Trichothecium roseum*, were of relatively weak action. The used candidates exhibited three mechanisms of action. The first of all was the production of antifungal compounds which inhibited *A. alternata* growth in dual cultures. This mechanism was reported by some other authors for some of these candidates (Dickinson *et al.*, 1995; Gupta *et al.*, 1995; Santamarina *et al.*, 2002). The second mechanism was mycoparasitism, that performed by *Trichoderma* species and *Trichothecium roseum*. The third mechanism was the competition for space, and may be for nutrient; this was shown by the fast growing fungus *Trichoderma*.

Efficiency of these candidates as biocontrol agents was measured using four preparations: (a) conidia alone, (b) conidia plus the growth medium, (c) conidia plus

mycelium, and (d) conidia plus the growth medium plus mycelium. All preparations reduced significantly the DI%, with the fourth preparation being the most efficient. In agreement with Mao *et al.* (1997), some preparations of *Trichoderma* were superior to the routinely used fungicide (Ridomil-Mancozeb). *Trichoderma* species (viz. *T. harzianum* and *T. viride*) were the most potent candidates followed by *Penicillium oxalicum*; both genera were exploited frequently as biocontrol agents for several other pathosystems (De Cal *et al.*, 1997; Lo *et al.*, 1997; De Cal *et al.*, 2000; Kumar and Kumar, 2000; Luz, 2001). *Aspergillus ustus* and *Trichothecium roseum* were less effective than the other candidates, nevertheless, they still as effective as the fungicide "Ridomil-Mancozeb". Establishment of a successful biological control system depends upon the construction of antagonistic population on and/or near the organs liable to infection. In this context, the fourth preparation could permits massive colonization of potato leaves which in turn leads to construction of antagonistic community. This may contribute to the great potency offered by the fourth preparation.

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## المقاومة غير الكيميائية لمرض اللفحة المبكرة في البطاطس الناتج عن فطر الألترناريا ألترناتا

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### الملخص العربى

يعد مرض اللفحة المبكرة في البطاطس أحد العوامل المؤثرة على إنتاج البطاطس. إن استخدام المقاومة الكيميائية لتقليل شدة المرض يمثل خطراً آخر على الزراعة. تعتبر المقاومة البيولوجية باستخدام الكائنات الدقيقة، وكذلك استخدام المستخلص المائي لكل من النبات والكمبوست من الوسائل الآمنة للحصول على مقاومة فعالة. يقلل تطبيق أى من هذه الطرق من نسبة حدوث المرض بما يصل إلى أقل من 2% وذلك خلال موسمين متتاليين. لقد أدى رش جراثيم الكائنات الدقيقة على نباتات البطاطس المريضة إلى تقليل المرض بنسب مختلفة، ولقد لوحظ أن فطر التريكودرما (*Trichoderma*) كان الأكثر فاعلية من بين الكائنات التي اختبرت ويليها فطر البنيسليوم (*Penicillium*). على الوجه الآخر، كان مستخلص الكحول لثلاث نباتات أكثر تأثيراً من المستخلص المائي لهذه النباتات، بينما كان كلا المستخلصين لنبات البوليجونم (*Polygonum*) أكثرهم تأثيراً في تثبيط حدوث المرض. كان المستخلص المائي للكمبوست ذو تأثير فعال ضد حدوث مرض اللفحة المبكرة عندما استخدم كرش على النباتات المصابة. كانت النتائج المتحصل عليها مقارنة لفعل المبيد الفطري المستخدم لمقاومة هذا المرض.