EFFECT OF RAPE AND MUSTARD SEED MEALS ON VERTICILLIUM WILT OF PEPPER

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Summary

The effect of plant material from Brassicaceae plants - the mustard seed meal and rapeseed meal, added to soil or peat substrate, on Verticillium wilt of pepper was evaluated in laboratory and greenhouse conditions. It was stated that the addition of these materials decreased infestation of pepper vascular vessels caused by Verticillium dahliae. The decomposition of rapeseed meal and mustard seed meal increased concentration of ammonia, the compound toxic to many pathogens. The increase of total bacteria, actinomycetes, spore forming bacteria were also observed. The materials from Brassicaceae plants positive influenced on plant development and chlorophyll content in pepper leaves.

key words: mustard seed meal, rapeseed meal, Verticillium dahliae, pepper

INTRODUCTION

Verticillium dahliae Kleb. has a wide host range and causes stuntling, wilting and reduction of plant growth and yields of many woody and herbaceous plants, e.g. tomato, eggplant, pepper, cruciferous plants (Klosterman et al. 2009). V. dahliae microsclerotia, a darkly pigmented resting structure, can survive in soil for many years and are the primary source of inoculum for V. dahliae infection in host plants (Xiao et al. 1997). Conidia or mycelium do not survive for a long time. After germination of microsclerotia on plant roots, the fungus penetrates them and invades the vascular tissues. An infection can occur at any time during the crop growth (Xiao & Subbarao 2000). In field soil microsclerotia of V. dahliae occur in clustered or aggregated patterns (Xiao et al. 1997).

Severity of Verticillium wilt is related to the inoculum population in soil at the beginning of growing season. According to Tjamos et al. (2000) biocontrol strategies against V. dahliae should be focused on reduction in survival of microsclerotia formed in plant tissues and present...
in soil, prevention of root infection by the fungus, and inhibition of microsclerotia formation on diseased plant tissues. The most effective method of controlling Verticillium wilt is fumigation of soil with chemical sterilants such as dazomet or metam sodium (Ślusarski 2008). Furthermore, these methods are efficient in decreasing amount of microsclerotia. These compounds however, kill microorganisms beneficial for plants, what often leads to increased populations of other pathogens due to reduced competition and antagonisms (Duniway 2002).

At present, to protect environment, other methods for reducing Verticillium propagules in soil or peat medium become increasing interest. One of the management strategies that can be useful in controlling *V. dahliae* is the application of organic amendment, i.e. the use of residues of plants containing biologically active compounds (Lopez-Escudero et al. 2007). The effectiveness of plant material from *Brassicaceae* and *Solanaceae* plants and antagonistic microorganisms, on survival of microsclerotia and development of Verticillium wilt of eggplant were discussed by Smolińska & Kowalska (2008). The authors observed that the addition of rapeseed meal, water extracts from rapeseed meal or tomato plants, significantly decreased number of *V. dahliae* microsclerotia in the soil. Studies of Brown & Morra (1997), Smolińska & Horbowicz (1999) have shown that the addition of *Brassicaceae* residues to soil decreased the population of soil borne fungal plant pathogens. Shetty *et al.* (2000) have attributed the inactivation of *V. dahliae* microsclerotia to toxic products of glucosinolate degradation released from cruciferous residues e.g. broccoli. Already it was observed that the application of high N-containing organic amendments to soil reduced the incidence of Verticillium wilt of tomato (Lazarovits *et al.* 2000).

The addition of organic amendments almost always caused an increase in population of microorganisms in soil. However, usually during a short period of time, their amount decreased to the initial level - the time when simple organic compounds, as sugars, organic acids, proteins, are used up. Some of these microorganisms may restrict population of pathogens in soil environment through direct antagonisms (antibiosis, parasitism), induction of resistance against pathogens or growth promoting activity (Harman 2004). Some attempts to decrease the number of *V. dahliae* propagules by use of selected antagonistic bacteria or fungi were done, but usually their efficiency was not stable.

In the present work we used rapeseed meal and milled seeds from mustard to control Verticillium wilt on pepper plants. These materials, especially rapeseed meal, are produced in huge quantity during production of oil and are cheap and easy accessible. In this paper we try to explain how rape and mustard seed meals may protect pepper against *Verticillium dahliae*.

**MATERIAL AND METHODS**

**Organic material from *Brassicaceae* plants.** Rapeseed meal (RSM) as the commercial product (Ardex, Poland),

**Pathogen.** *Verticillium dahliae* was isolated from diseased pepper plants and maintained on potato dextrose agar medium (PDA-Merck) in Microbiology Lab. of the Research Institute of Vegetable Crops. To produce microsclerotia, *V. dahliae* was cultivated in the liquid glucose-mineral salt medium (Dhingra & Sinclair 1995) or on the Czapek-Dox agar with cellophane (Kowalska & Smolińska 2003).

**The evaluation of Verticillium wilt severity - greenhouse experiment.** Peat substrate (Klasmann) was divided into equal - 50 L parts. To each part 500 mg of *V. dahliae* microsclerotia were added and mixed thoroughly for about 10 min. One part without the fungus served as a control. Then, 250 g of rapeseed meal (RSM) or milled seeds of mustard (seeds were milled in blender for about 20 sec) were added and mixed. Nemazin 97 XX (a.i. dazomet) added in amount of 12.5 g per 50 L of peat substrates served as a chemical control. All parts were watered with tap water to receive about 60% humidity. The substrate was put into plastic bags, covered to avoid drying, but to also enable air penetration. After a 2-month incubation at 18-20ºC, the substrate from plastic bags was placed into 5 L pots (10 pots for one treatment) and two-month old seedlings of pepper cv. Ożarowska were transplanted into each of them. The plants were grown in greenhouse and were managed according to the standard agricultural practice, including recommended nutrition, irrigation and plant protection. At the end of the vegetation period of pepper, each plant was cut across main stalk. The pepper plants were cut to the following size: 1/ - 5 cm above level of substrate; 2/ - before first branch; 3/ - 10 cm from the end of the highest stalk. The level of browning of vascular vessel was evaluated in the rates: 0 - plant healthy, 5 - all vessels dark-brown. The experiments were conducted in the years 2008 and 2009.

**Chlorophyll content - greenhouse experiment.** The measurement of chlorophyll content index was conducted in three leaves from each plant (30 measurements per one treatment at the time), using CCM-200 Chlorophyll Content Meter. The leaves were taken just above the first branch. Measurements of chlorophyll content were conducted 4, 6, 8 and 12 weeks after transplanting the pepper seedlings to the 5 L pots.

**Changes in the concentrations of nitrogenous compounds.** The experiment was conducted in laboratory conditions. To the field soil and peat substrates (the chemical composition is presented in Table 3) the rapeseed meal and mustard seed meal at the dose of 0.5% w/w was added. Three plastic containers (3 L volume) per plant, covered with a lead with a small opening (0.5 cm diameter), contained 3 kg of soil or 2 kg of peat substrates. The moisture was kept at about 60%. The soil samples for chemical analysis were taken after 24 hours, one week and one month after incubation at room temperature (22-24ºC). The amount of inorganic N-compounds (total N; NH$_4^+$; NO$_3^-$) during degradations of rapeseed meal and mustard seed meal in soil and peat...
substrates was evaluated. The experiments were conducted twice.

To evaluate the concentration of nitrogen compounds, samples N-NH$_4^+$, N-NO$_3^-$, were extracted with 0.03N acetic acid from soil or peat medium in proportion: 1:10 (Nowosielski 1988). Both forms of nitrogen were determined with calorimetric method using continuous flow analyser (San Plus, Skalar). Total N was determined with Kjeldahl method, and pH in the distilled water in the proportion: 1 part medium/2 parts of water.

**Microbial analysis:** To study the microbial populations in the soil or peat substrates, to which organic compounds were added, the following media were used: soil extract agar (SEA) for evaluation of the total bacteria and Actinomycetes; tryptose soya agar (TSA) for spore forming bacteria (the diluted suspensions were heated to 80ºC for 10 min.) (Dhingra & Sinclair 1995); whereas Gould medium (Gould et al. 1985) for fluorescent Pseudomonas enumeration. The number of fungi in the soil was evaluated on rose bengal medium (Martin 1950). The analysis of microbial populations were conducted 30 days after addition of plant material. The number of microorganisms was expressed as number of colony forming unit (cfu)·1 g$^{-1}$ of soil dry weight.

**Chemical analysis of plant leaves.** After drying at 65ºC the plant material was ground and subjected to mineralization with concentrated nitric acid using the Milestone microwave oven, model Ethos-1. The total N was determined using Kjeldahl’s method. The other nutrient elements were determined at different wavelengths using Perkin-Elmer ICP-spectrometer model Optima 2000DV.

**Statistical analysis.** All experiments conducted in this study were repeated at least once. The data were analyzed by standard statistical procedure. Significance of differences between means was established by analysis of variance and Newman-Keuls test at $P=0.05$.

**RESULTS AND DISCUSSION**

The results obtained from previous work (Smolińska & Kowalska 2008) showed that the plant residues from Brassicaceae plants decreased survival of *V. dahliae* microsclerotia in the laboratory conditions. The results obtained in this study showed that the addition of rapeseed meal (RSM) or mustard seed meal to peat substrates decreased infestation of pepper plant vessels with *V. dahliae* (Table 1). The big differences of plant infestation within treatments caused the results were statistically insignificant. Similar effect was observed in the experiments with eggplant, where the addition of rapeseed meal to peat substrates hampered development of Verticillium wilt of these plants (Smolinska & Kowalska 2008). However, similarly to the eggplant, the colonization of vascular vessels in the plant shots was the lowest in the plants growing in the substrate with Nemazin.
Table 1. Infestation of vascular vessels in the pepper growing in peat substrates infested with *Verticillium dahliae* microsclerotia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Crossection 1**</th>
<th>Crossection 2**</th>
<th>Crossection 3**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (without pathogen)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>1.10</td>
<td>0.80</td>
<td>0.5</td>
</tr>
<tr>
<td>Nemazin 97 XX + <em>V. dahliae</em></td>
<td>0.20</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>RSM+ <em>V. dahliae</em></td>
<td>0.70</td>
<td>0.35</td>
<td>0.0</td>
</tr>
<tr>
<td>Mustard+ <em>V. dahliae</em></td>
<td>0.25</td>
<td>0.10</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* The level of browning of vascular vessel was evaluated in the rates: 0 - vessels without infestation, 5 - all vessels dark-brown. **The crossection were made: 1/ - 5 cm above level of substrate; 2/ - before first branch; 3/ - 10 cm from the end of the highest stalk. The results in the columns were insignificant according to Newman-Keuls test at P=0.05.

Undoubtedly the mechanism of detrimental effect of *Brassicaceae* plant amendments on development of *Verticillium* wilt is very complex. It is known that during decomposition of organic material in soil many phytotoxic compounds are released, especially during the first days after application. Rapeseed and mustard meals used in these experiments contained biologically active compounds - glucosinolates. Rapeseed meal contained 17.6 µM·g⁻¹ of glucosinolates (results not presented). In this work the level of glucosinolates in mustard was not analyzed. However, judging from our previous study conducted with mustard (B. *juncea* cv. Malopolska), the concentration of glucosinolates in tissues of these plants varied from 34.39-50.15 µM·g⁻¹ (Smolińska 2004). During decomposition of rapeseed or mustard seed meal in soil or peat medium glucosinolate degradation products, isothiocyanates, thiocyanates, nitriles, are released. Detrimental effect of *Brassicaceae* residues on microorganisms has attributed to these degradation products, mainly highly toxic isothiocyanates. Antimicrobial properties of these compounds are evident and proved in many publications (Kanemaru & Miyamoto 1990; Brabban & Edwards 1995; Manici *et al.* 1997). However, Mazzola *et al.* (2001) showed that suppression of specific apple root pathogens by *Brassica napus* seed meal occurred regardless of content of glucosinolates in RSM. It is obvious that fungitoxic effectivity of glucosinolate degradation products depends strongly on concentration of these compounds in soil microsites and it is connected with many other factors, for example: water content, pH, organic matter (Brown & Morra 1997). During decomposition of rapeseed and mustard seed meals a lot of active volatile and non volatile compounds, detrimental towards microorganisms, are produced. Depending on conditions prevalent in soil or peat substrates, and concentration of the compounds, it may act as fungitoxic or fungistatic. As it was observed in previous research detrimental activity of compounds may make fungal propagules more vulnerable to attack by soil microorganisms (Smolińska 2004).
Table 2. Content of micro- and macroelements in rapeseed (RSM) and mustard seed meals

<table>
<thead>
<tr>
<th>Organic material</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
<th>Fe</th>
<th>Mn</th>
<th>Cu</th>
<th>Zn</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mustard seeds</td>
<td>48.5</td>
<td>5.80</td>
<td>7.96</td>
<td>3.41</td>
<td>5.53</td>
<td>127</td>
<td>28.4</td>
<td>40.1</td>
<td>55.0</td>
<td>52.0</td>
</tr>
<tr>
<td>RSM</td>
<td>52.0</td>
<td>8.24</td>
<td>12.3</td>
<td>5.01</td>
<td>7.71</td>
<td>139</td>
<td>65.6</td>
<td>12.3</td>
<td>73.7</td>
<td>42.3</td>
</tr>
</tbody>
</table>

Both materials, rapeseed meal and mustard seed meal, contained considerable quantity of nitrogenous compounds (Table 2). Results obtained in this work showed that during decomposition of RSM and mustard seed meal in soil and peat substrates, considerable quantity of ammonia NH₃ are formed (Fig. 1 & 2). The process of formation ammonia started immediately after addition of plant material. Its concentration quickly increased during a few days and decreased to the starting point during the month (Fig. 1 & 2). Ammonia is the toxic compound for microorganisms, though for technical limitations in this work the concentration of ammonium ion (NH₄⁺) had been determined. It is known (Tenuta & Lazarovits 2002a) that rate of ammonia (ammonium) formation is strongly dependent on many factors as: pH, organic matter, moisture content. The ratio of ammonia to ammonium depends on pH. As pH increases, ammonium (NH₄⁺) is converted to ammonia (NH₃). Soil pH at or above 8.0 initiates the conversion of NH₄⁺ into NH₃ with the equilibrium (pKₐ) between ammonium and ammonia occurring at pH 9.3 (Klosterman et al. 2009). In presented work, the level of NH₄⁺ was measured. As the dissociation of ammonium in alkaline soil to form ammonia is faster than in acid environment it is probable, that at least at the beginning of decomposition of plant materials, considerable quantity of NH₃ was formed. Ammonia, near NO₂⁻ is one of the most toxic compounds formed in soil during degradation of organic materials containing high concentration of nitrogen.

Table 3. Chemical composition of field soil and peat substrates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Soluble salts g dm⁻³</th>
<th>N(NO₃)</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field soil</td>
<td>7.7</td>
<td>0.54</td>
<td>72</td>
<td>101</td>
<td>262</td>
<td>168</td>
<td>2592</td>
</tr>
<tr>
<td>Peat substrates</td>
<td>5.8</td>
<td>1.78</td>
<td>213</td>
<td>140</td>
<td>233</td>
<td>297</td>
<td>1344</td>
</tr>
</tbody>
</table>

Soil and peat medium used in the presented work, differed significantly in chemical composition (Table 3). However, in both cases the highest concentration of ammonium were observed in treatment with RSM (Fig. 1 & 2). In soil this compound was formed more quickly than in peat medium, but in soil NH₄⁺ fast converted into NO₃⁻. Nitrate contrary to nitrite is non toxic towards microorganisms.
Fig. 1. Changes of total nitrogen $\text{N}\cdot\text{NO}_3^-$, $\text{N}\cdot\text{NH}_4^+$ and pH occurring in field soil after addition of RSM and mustard seeds.

Fig. 2. Changes of total nitrogen $\text{N}\cdot\text{NO}_3^-$, $\text{N}\cdot\text{NH}_4^+$ and pH occurring in peat substrates after addition of RSM and mustard seeds.

After one month, the concentration of ammonium decreased to the “control” level, so it is possible that first days after addition of RSM and mustard seeds meal are the most detrimental to $V.\ dahliae$. Similar
observation had Conn et al. (2005) after application of liquid swine manure to soil. He concluded that ammonia released from liquid swine manure could kill V. dahliae microsclerotia in soil together with volatile fatty acid and nitrous acid (Conn et al. 2005). Tenuta & Lazarovits (2002a, 2002b) demonstrated that ammonia and nitrous acid were lethal to V. dahliae microsclerotia in soil receiving meat residues and bone meal and synthetic (urea) nitrogen amendments. Lazarovits et al. (2000) observed, that the efficacy of an amendment in controlling V. dahliae varied with the soil tested. It is important to note that fungitoxic effect of ammonia or glucosinolate degradation products, is dependent on adequately high concentration of these compounds in the environment surrounding the pathogen. Because distribution of plant residues in soil or peat substrates is not uniform, harmful effect on pathogen population may be vary a lot from site to site, even within small distances.

Many different compounds are formed during decomposition of organic material in soil. Most of these compounds, mainly fast decomposable sugars and organic acids are very good sources of food for microorganism. The rate of decomposition of plant tissues, and formation from complex compounds like proteins or nucleic acids, simple compounds (ammonia), also depend on activity of microorganisms in environment (Paul & Clark 2000). In our experiment, bigger amount of bacteria at the beginning of the experiment (compared to peat) was observed in soil, and in consequence, quicker decomposition of organic compounds occurred.

![Figure 3](image)  
**Fig. 3.** Effect of RSM and mustard seed meal on population of microorganisms in soil and peat substrates
The addition of rapeseed or mustard seed meal altered communities of soil microorganisms. The results obtained in this work showed that the addition of rapeseed residues caused significant increase in population of total bacteria, *Actinomycetes* and spore forming bacteria (Fig. 3). They possess many features that make them well suited as biocontrol agents (Weller 2007). These include the ability to effective colonization and multiply in the rhizosphere and production a wide spectrum of bioactive metabolites (McSpadden & Gardener 2007). These features enable them to compete aggressively with pathogenic microorganisms. It is difficult to speculate if this activity towards *V. dahliae* was formed through direct action of antagonistic bacteria (or fungi) or indirectly through protection of plant roots. Also Tjamos (2000) and Tjamos *et al.* 2000 demonstrated that antagonist belonging to the spore forming, Gram positive bacteria from the *Bacillus* sp. group, were able to reduce the germination of microsclerotia up to 60% when applied as a soil drenching suspension. Other authors reported antagonistic properties of *Bacillus* and *Pseudomonas* bacteria towards *V. longisporum* (Berg & Lottman 2000).

As demonstrated by Huisman and Gerik (1989) root tips and elongation zone are the sites of host invasion by *V. dahliae*. Prevention of germination of microsclerotia in root tips and elongation zone by fungal or bacteria antagonists was demonstrated. Many microorganisms that occur naturally in the rhizosphere have the ability to interfere with pathogen growth, survival or infection. There are also several mechanisms of these actions, for example, competition for space or nutrients on the root surface, mycoparasitism or the release of toxic compounds (Borneman & Becker 2007). During this work the analysis of population of selected group of microorganisms on the roots of pepper growing in medium with and without *Brassicaceae* material were conducted. The higher level of spore forming bacteria and fungi in the rhizosphere of pepper growing in peat medium amended with rapeseed and mustard seed meals compare to the pepper growing in substrate without addition was observed (results not presented). Therefore, it is possible that more microorganisms antagonistic to the pathogen, that prevent germination of microsclerotia, were present in the rhizosphere of host plants. It is possible that the higher level of microorganisms, which was observable after addition of *Brassicaceae* residues, could suppress pathogen activity via antibiosis or competition. It is unclear to what degree these bacteria contribute to pathogen suppression, especially that multiple abiotic and biotic factors could affect the abundance and activities of specific bacterial populations. The studies of Johansson and Wright (2003) dealt with seedling blight caused by *Fusarium culmorum*, showed that disease-suppressive bacteria were isolated more frequently from plants belonging to the family *Brassicaceae* than from the other plants.
Table 4. Chemical content of pepper leaves (the analysis were done between first and second chlorophyll measurements)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N&lt;sub&gt;NO₃&lt;/sub&gt;</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10160</td>
<td>6100</td>
<td>93240</td>
<td>8317</td>
<td>3170</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>10370</td>
<td>6200</td>
<td>99450</td>
<td>8252</td>
<td>2910</td>
</tr>
<tr>
<td>Nemazin 97XX+<em>V. dahliae</em></td>
<td><strong>11670</strong></td>
<td><strong>7300</strong></td>
<td>78210</td>
<td>7204</td>
<td>2880</td>
</tr>
<tr>
<td>RSM+<em>V. dahliae</em></td>
<td><strong>13180</strong></td>
<td><strong>6400</strong></td>
<td>71280</td>
<td>8333</td>
<td>3110</td>
</tr>
<tr>
<td>Mustard + <em>V. dahliae</em></td>
<td><strong>11150</strong></td>
<td><strong>7000</strong></td>
<td>74440</td>
<td>6968</td>
<td>3680</td>
</tr>
</tbody>
</table>

Fig. 4. Changes in chlorophyll content in the leaves of pepper growing in peat substrates amended with RSM and milled seed of mustard infested with *V. dahliae*

Another effect of amendments of peat substrates with rapeseed meal or mustard meal seeds is the positive effect on plant growth. The plants growing in the substrates with RSM and mustard seed meal were greener and looked better than in the control treatment. They had higher chlorophyll content than plants from control treatment (without pathogen) and with *V. dahliae* only (Fig. 4). The results were confirmed by chemical analysis. The pepper plant leaves had higher concentration of nitrogen and P in the leaves than in the treatments without organic additions (Table 4). An interesting fact is that pepper growing in peat substrates with Nemazin 97 XX also had higher level of N<sub>NO₃</sub> and P. In this case it may have been one of...
two causes: first, fumigation with Nemazin immobilized organic compounds coming from killed organisms, second - plants were more healthy.

In conclusion, from results obtained from this and previous studies we assumed that after addition of rapeseed meal or mustard seed meal there were at least 4 mechanisms which might influence on population of *V. dahliae* in soil or peat substrates and severity of plant disease (Fig. 5). The population of *V. dahliae* in soil or peat substrates could be decreased through toxic activity of compounds released during decomposition of plant material, as well as simple compounds as ammonia or degradation products of glucosinolates as isothiocyanates, nitriles or thiocyanates. Microorganisms which developed intensively after addition of plant material might act either directly on microsclerotia and mycelium (antagonistic or parasitic activity) or on plants, stimulating their growth. Development of disease is the consequence of many factors among which the quantity of pathogen propagules and plant vigour are the most important.

![Control of Verticillium wilt by rapeseed meal or mustard seed meal](image)

**Microsclerotia are killed by ammonia (or nitrous acid)**

**Increase population of microorganisms antagonistic to *V. dahliae***

**Microsclerotia are killed by degradation products of glucosinolates released from *Brassicaceae* residues**

**Effect of organic amendments on plant growth**

Fig. 5. The possible ways of action of *Brassicaceae* plant material on Verticillium wilt

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Tenuta M., Lazarovits G. 2002a. Identification of specific soil properties that affect the accumula-


ODDZIAŁYWANIE WYTŁOCZYN Z RZEPAKU I MĄCZKI Z NASION GORCZYCY NA WERTYCYLOZĘ PAPRYKI

Streszczenie

W warunkach laboratoryjnych i szklarniowych badano wpływ mączki z nasion gorczycy i wytłoczyn z rzepaku, dodanych do gleby lub podłoża torfowego, na rozwój wertycyliozy papryki. Stwierdzono, że dodatek tych materiałów zmniejszał porażenie wiązek przewodzących papryki przez *Verticillium dahliae*. Rozkład wytłoczyn z rzepaku i mączki z nasion gorczycy w glebie i podłożu torfowym zwiększał stężenie amoniaku, związku toksycznego dla wielu patogenów. Obserwowano także wzrost ogólnej liczebności bakterii, bakterii przetrwałnikowych oraz promieniowców. Materiały z rzepaku i gorczycy korzystnie wpływały na kondycję roślin papryki i zwiększały zawartość chlorofilu w liściach tej rośliny.

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