ARTICLE; AGRICULTURE AND ENVIRONMENTAL BIOTECHNOLOGY

Effect of different soil amendments on the microbial count correlated with resistance of apple plants towards pathogenic Rhizoctonia solani AG-5

Ebaa Ebrahim El-Sharouny*

Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt

(Received 3 October 2014; accepted 27 October 2014)

In this study, a trial was made to control the infection of apple plants by Rhizoctonia solani AG-5. Five soil amendments: compost, cow manure, chicken manure, Brassica juncea seed meal and urea, were applied to Columbia View Experimental soil planted with six-week-old apple transplants. Soil was applied in two forms, pasteurized and non-pasteurized. The results showed that the amendments enhanced the increase in microbial count. The effect was more pronounced in fungi and pseudomonads. The increase in the fungal count was particularly clear in the case of B. juncea seed meal and urea, leading to 40- and 20-fold increase, respectively. Regarding the pseudomonads count, the same two amendments, B. juncea seed meal and urea, resulted in 76.5- and 54-fold increase, respectively. It was also observed that apple seedlings planted in pasteurized soil started to wilt and die faster than those planted in non-pasteurized soil, regardless of the type of amendment supplied. Brassica juncea seed meal showed the most pronounced positive effect when added to non-pasteurized soil, which could completely prevent the infection of apple seedlings. In tests to detect the DNA of R. solani AG-5 in the roots of apple replants by real-time polymerase chain reaction, it was observed that only B. juncea seed meal and urea could significantly reduce the pathogen DNA level in the pasteurized soil and decreased it to a zero level in case of non-pasteurized soil.

Keywords: soil amendments; apple seedlings; microbial count; Brassica juncea; Rhizoctonia solani

Introduction

Fungal plant pathogens are among the most important factors that cause serious losses to agricultural products every year.[1] Biological control of plant diseases including fungal pathogens has been considered a viable alternative to chemical control which has caused a number of ecological concerns during the last four decades. In addition, biocontrol agents are easy to deliver, improve plant growth, activate the resistance mechanisms in the host and increase the biomass production and the yield.[2,3] Interactions between plants and pathogens can affect plant health in various ways. There are different mechanisms of action by which biocontrol-active micro-organisms can control fungal plant diseases (reviewed in [1]). These mechanisms include hyperparasitism, predation, antibiotics, cross-protection, competition for sites and nutrients and induced resistance.[4,5]

Biocontrol micro-organisms can also be used in the form of compost in some plants. Addition of composted organic matter to potting mixes has been shown to result in suppression of soil-borne diseases.[1] There are also soil amendments other than composts that show a significant improvement in the resistance mechanisms of plants against pathogens. Such amendments are, for example, mustard (Brassica juncea) and flax (Linum usitatissimum) seed meals and sorghum-sudangrass (Sorghum bicolor) added to the soil at appropriate levels.[6,7] Mustard meal can considerably affect the microbial community composition, appearing to select for specific fungal populations. [6] Mazzola and Brown [8] studied the efficacy of brassicaceous seed meals for control of apple replant disease and the effects of such treatments on the causal pathogen complex including Rhizoctonia, Pythium and Phytophthora.

An amendment is considered effective when it can create a soil environment that is effective against the target pathogen and at the same time has a favourable effect on the activity of beneficial micro-organisms, on soil fertility and on plant growth. In other words, the purpose of soil amendments is to reinforce the harmful effects on target pathogens and maintain — and even enhance — soil fertility with the least possible negative impact on agroecosystems.[1]

Materials and methods

Orchard soils

Soil was collected from the Columbia View Experimental (CV) Orchard, Orondo, WA. The characteristics of this
soil, both physical and biological, are described in [9]. Soil pH ranged from 6.9 to 7.1.

**Soil amendments**

Soil amendments used in this study include urea, seed meals derived from *B. juncea*, chicken manure, cow manure and compost. These amendments were added as supplements to soil at a concentration of 0.5% (wt%).

**Media**

Trypticase soy agar was used for scoring bacterial counts. Cycloheximide was dissolved in H$_2$O and was added at a concentration of 750 mg L$^{-1}$. Potato dextrose agar was used to determine the fungal counts. Kanamycin (750 mg L$^{-1}$ H$_2$O) and streptomycin (1000 mg L$^{-1}$ H$_2$O) were added after autoclaving. King’s medium [10] was used for isolating and counting *Pseudomonas*. King’s medium was amended with ampicillin (1000 mg L$^{-1}$ H$_2$O), chloramphenicol (130 mg L$^{-1}$ H$_2$O) and cycloheximide (750 mg L$^{-1}$ H$_2$O).

**Maintenance of Rhizoctonia solani AG-5**

*Rhizoctonia solani* AG-5 was grown on oat seeds previously autoclaved twice, then left for one month and sieved to obtain fine-sized particles.

**Microbial counts in soil**

At the beginning of the experiment, the total microbial count was determined in each type of soil (pasteurized or non-pasteurized, amended or non-amended), using the above-mentioned types of media. Each type of soil was kept in a refrigerator if not used directly.

**Effect of soil amendments on Rhizoctonia solani AG-5 apple root infection**

Twelve kilograms of CV Orchard soil were used in this study. Half the amount was pasteurized twice in an oven at 90 °C for over one day. *Rhizoctonia* grown on oat seeds was added to both pasteurized and non-pasteurized soils at a rate of 0.5% (wt%) and left for 24 h. Later, each soil sample was divided into six portions, each portion receiving a different amendment. The amendments were left for one week in the soil before cultivation of apple transplants. Apple transplants at an age of six weeks were used. Each treatment was applied in seven replicates and was watered directly with 10 mL of tap water. Transplants were randomized in their places and then the whole tray was placed in a growth chamber at 24 °C for 14 h and 16 °C for the remaining 10 h. The rating of the results was taken after three and six days. After six days from transplanting, all plants were uprooted. Replicates of each treatment were collected as a bulk where three samples were taken from the bulk roots for the real-time polymerase chain reaction (PCR) experiment.

**Real-time polymerase chain reaction**

Real-time PCR was used to detect *R. solani* DNA.[8] DNA was extracted from *R. solani* AG-5 1007 mycelium, using an MO BIO UltraClean Microbial DNA isolation kit (MO BIO Laboratories, Carlsbad, CA), and amplified with ITS 4 and ITS 5 primers. It was reamplified with RSAG5 F and RSAG5 R primers, using a GeneAmp® PCR System 9700 (Applied Biosystems), and finally, with the same primers, using real-time PCR.

**Characterization of DNA from Gala apple seedlings**

DNA from Gala apple seedlings grown in soil infested with *R. solani* AG-5 was extracted using the MO BIO UltraClean Plant DNA isolation kit (MO BIO Laboratories, Carlsbad, CA). DNA was extracted from a 50 mg of fine root sample from each treatment.[8]

**Results and discussion**

Plants possess inducible defence mechanisms that are activated upon contact with pathogenic or non-pathogenic micro-organisms, extracts of micro-organisms or chemicals, thus providing protection against a broad spectrum of pathogens.[3]

**Effect of different amendments on soil microbial population**

Organic material amendments (e.g., manure, compost, plant residues) to the soil have been shown to affect soil microbial populations and soil suppressiveness by promoting beneficial micro-organisms native to the soils and/or by introducing new beneficial micro-organisms.[11,12] All of these micro-organisms work on the mineralization of organic matter in soil. The results presented in Table 1 show that, in general, the microbial count in non-pasteurized soil was significantly higher than that in pasteurized soil in the range from 1.4- to 80-fold. Regarding the effect of the added amendments, they notably enhanced the increase in microbial count. The effect was more pronounced in fungi and pseudomonads as compared with bacteria. The increase in the fungal count was higher in the case of *B. juncea* seed meal and urea, with an increase from 20 to 800 CFU g$^{-1}$ (40-fold increase) and from 9 to 180 CFU g$^{-1}$ (20-fold increase), respectively. The same two amendments, *B. juncea* seed meal and urea, resulted in, respectively, a 76.5-fold and 54-fold increase in the count of soil pseudomonads.
It has been suggested that soil type is a key determinant for soil microbial activity and community structure. [13] Mandic et al. [14] evaluated the effect of application of different rates of mineral nitrogen, well-rotten farmyard manure and Klebsiella planticola SL09-based microbial biofertilizer on the count of soil micro-organisms during the cultivation of potato plants. Their results suggested that well-rotten farmyard manure induces the highest increase in microbial counts, potato yield and stem height. Another report, that of Zaccardelli et al. [15], investigates the effects of increasing doses of compost on the density of several cultivable soil microbial groups in an agricultural system in Southern Italy. It shows that compost furnishes a high number of spore-forming bacteria exhibiting antibiotic activity against phytopathogenic fungi. Another study, by Lin et al. [16], focuses on the effects of different organic fertilizers (chemical fertilizer, pig manure, cattle manure, organic compound fertilizer of monosodium glutamate and chicken manure) on soil microbial biomass and peanut yield. The total amounts of bacteria, epiphytes and actinomycetes were highest in the variant with application of chicken manure as compared with different fertilizer treatments.

Effect of soil amendments on the incidence of Rhizoctonia solani AG-5 infection in apple roots

Organic soil amendments have commonly been used to provide for plant nutrition and control of soil-borne plant pathogens. Composts have been used extensively in high value cropping systems, and it is believed that the control of certain pathogens, such as Pythium spp., is brought about through an overall increase in microbial activity, typically referred to as general suppression.[17] However, there are data suggesting that the microbial complex introduced through compost amendment is less effective in some soil systems than the resident microbial community when such materials are evaluated in natural disease-suppressive soils.[18] So far only few studies have shown that soil amendments not only affect suppressiveness to soil-borne diseases, but also the resistance of host plants to airborne diseases.[3,19] It has been demonstrated that organic amendments can be very effective in controlling diseases caused by fungal pathogens.[20,21] Different complementary mechanisms have been proposed to explain the suppressive capacity of organic amendments: enhanced activities of antagonistic microbes,[22] increased competition against pathogens for resources that cause fungistasis, [23] release of fungitoxic compounds during organic matter decomposition [24] or induction of systemic resistance in the host plants [25,26]. For review see [27].

In this study, different amendments were added to pasteurized and non-pasteurized soils to study their effect on the incidence of infection of apple roots by R. solani, where the rating of symptoms was recorded three and six days after planting. It was observed that apple transplants planted in pasteurized soil started to wilt and quickly die faster than those planted in non-pasteurized soil. Regardless of the type of amendment supplied to the non-pasteurized soil, all transplants were either wilted or dead six days after being planted (Figure 1). This could be explained by the reduced microbial count or absence of microbial population in pasteurized soil which seems to be acting as a cooperating counterpart with the microflora in the added amendments. On the other hand, the non-pasteurized soil helped the amendments to show their positive impact on the plant health, with all the apple transplants being kept healthy for three till six days after planting in B. juncea-amended soil. Cow manure and urea showed the second best effect, with 71.14% of the plants remaining healthy after six days. In composted soil, there were 57.14% healthy transplants, in addition to 28.6% wilted ones, whereas only 14.26% were completely dead. Soil amended with chicken manure showed similar results as the non-amended (control) soil.

### Correlation between disease suppression and pathogen populations

According to Mazzola [28], real-time PCR can detect small quantities of target DNA from microbially complex environmental samples and has been used frequently in studies of disease risk assessment. However, this method has been criticized for being unable to discriminate viable from non-viable fungi or fungal structures, which might give information about the real threat for the plant (reviewed in [29]). This problem could be solved to some extent by amplification of fungal RNA using the reverse transcription PCR.[29]

In this study, Mazzola’s approach was followed by tracking the DNA of the pathogen R. solani in the roots of

| Table 1. Microbial counts in pasteurized and non-pasteurized soils supplemented with different amendments. |
|---------------------------------------------------------------|
| Type of soil | Amendment    | Fungi (CFU mg⁻¹) | Bacteria (CFU mg⁻¹) | Pseudomonads (CFU mg⁻¹) |
|---------------|--------------|-----------------|-------------------|------------------------|
| Pasturized    | Control      | 2 × 10³         | 98 × 10²           |                        |
|               | Urea         | 9 × 10³         | 121 × 10²          |                        |
|               | B. juncea    | 20 × 10⁴        | 68 × 10³           |                        |
|               | Chicken manure | 20 × 10³      | 93 × 10²           |                        |
|               | Cow manure   | 40 × 10³        | 135 × 10²          |                        |
|               | Compost      | 180 × 10³       | 113 × 10³          |                        |
| Non-pasteurized | Control     | 190 × 10³       | 40 × 10³           |                        |
|                | Urea         | 180 × 10³       | 65 × 10³           |                        |
|                | B. juncea    | 800 × 10⁵       | 52 × 10⁵           |                        |
|                | Chicken manure | 150 × 10⁴     | 38 × 10⁴           |                        |
|                | Cow manure   | 200 × 10⁴       | 57 × 10⁴           |                        |
|                | Compost      | 260 × 173 × 10³ | 125 × 10³          |                        |
Figure 1. Effect of adding amendments to pasteurized and non-pasteurized soils on the incidence of infection of apple roots by *Rhizoctonia solani* AG-5.
apple replants using the real-time PCR. It could be seen that there was high level of pathogen DNA in the roots of plants in pasteurized soil (Figure 2). The highest amount of *R. solani* AG-5 DNA was detected in the variants with addition of cow manure and chicken manure, while a moderate level was seen with the control and composted soils and the least amount, which was hardly detectable, with *B. juncea* seed meal and urea. Cow waste and chicken waste seem to encourage the growth of the pathogen instead of suppressing it. Many studies refer to the nitrogen content of organic amendments as the factor that increases the resistance of plants.[30] This suggestion is based on data that urea has the maximum nitrogen content (46%), followed by green manure (1.5%–5%), chicken manure (1.5%–3%), compost (0.5%–2%) and finally cow waste (0.3%–0.6%). However, the results from the this study could definitely confirm the role of nitrogen in affecting the presence of pathogens in the soil, as there is a significant difference in nitrogen content in both chicken and cow wastes. An alternative explanation could be sought in the native micro-organisms present in cow and chicken manure that could have no or, rarely, an antagonistic effect on the *Rhizoctonia* pathogen.

On the other hand, in the non-pasteurized soil, there was a very sharp reduction in the amount of pathogen DNA, which reached a zero level in the case of compost, *B. juncea* seed meal and urea, followed by chicken manure and even non-amended soil. The lowest effect was observed in the non-pasteurized soil amended with cow manure.

The obtained results based on the detection of *R. solani* AG-5 DNA are in agreement with the above observations of the incidence of infection in apple plants, where, in the non-pasteurized soil, especially that amended with *B. juncea* seed meal or urea, there was a low level of pathogen DNA and a low rate or complete absence of infection of plants after six days of cultivation. Moreover, the very fast wilting and death of apple plants in pasteurized soil with all amendments could also be considered as another supportive evidence for the high activity of the pathogen as evaluated at the DNA level, in addition to the great reduction in the count of the native microbial population by pasteurization.

Despite the potential value of organic soil amendments, there are, however, several concerns about its efficacy and potential side-effects that limit practical applications.[26] For example, organic amendment has, in some cases, variable effectiveness and may sometimes even aggravate disease severity.[11,31] Such negative effects of organic amendment are often associated either with increased inoculum of pathogenic fungi (since organic matter provides the substrate for their saprophytic growth) or with release of phytotoxic compounds that may damage plant roots and predispose them to pathogen attack.[26,27] One of these explanations could possibly be the reason behind the results about the effect of cow and chicken manure observed in the present work (Figure 2).

On the other hand, bioactive plant products which were originally introduced as soil amendments, have also acquired significant use as a disease control measure.[17] Members of the plant family Brassicaceae produce glucosinolates which, upon hydrolysis, yield biologically active compounds which have a broad spectrum of antimicrobial activity (isothiocyanates). Thus, these plants have
attracted attention as potential ‘biofumigants’, where incorporation of plant residue into soil ultimately results in the release of active hydrolysis products.[7,32] There is evidence that Brassicaceae plant residues may operate in the suppression of fungal pathogens via a different, as yet unidentified, mechanism.[33] Many reports confirm the effective use of *Brassicanapus* residues and *B. juncea* to control soil-borne plant diseases.[7,8,17] Likewise, the protective effect of Brassicaceae residues against soil-inhabiting fungal plant pathogens and also insect pests has been observed to increase for weeks after isothiocyanates are lost from the soil either by volatilization or degradation.[34,35]

It has been hypothesized [17] that the oxidation of nitrogen (N) in soil amendments to nitric oxide (NO) may be among the bacterial activities that contribute to disease suppression, since NO is known to stimulate certain plant defence pathways.[36,37] Evidence in support of this hypothesis can be traced back to five decades ago, when Huber et al. [38] reported a negative correlation between fungal root disease and the capacity of an amendment to stimulate nitrification. It is nitrifying bacteria that are now recognized as the major source of NO from soils.[39] In the light of these accumulating data, our study provides an additional insight into how efficient the organic soil amendments are, especially the Brassicaceae plant residues, in controlling apple plant diseases, either indirectly, while acting as a biofertilizer for the plant itself or directly, by suppressing the plant pathogens causing the disease. This idea could be generalized for reapplication on many other different economical crops, avoiding the unwise and overdose of chemical pesticides, and eliminating any risky consequences that may follow.

Conclusions

The data obtained in the this study suggest that organic soil amendments, especially the green manure derived from *B. juncea* seed meal, could be used as biocontrol agents, so they could significantly increase the resistance of apple plants towards soil-borne diseases. *Brassica juncea* and urea also proved to have a considerable efficacy as a means of eliminating and suppressing the apple pathogen *R. solani*. It could be speculated that one possible mechanism underlying this effect could be by increasing the total microbial count in the soil and, thus, increasing the antagonistic effect against the pathogen, or by production of substances with antimicrobial activity.

Acknowledgements

The author’s sincere thanks go to Dr. Mark Mazzola PhD (research plant pathologist) for kindly allowing the author to do this research in his lab at the USDA-ARS, Tree Fruit Research Laboratory, Wenatchee, WA, USA. The author is also grateful to Prof. Soraya A. Sabry (Professor of Microbiology, Faculty of Science, Alexandria University) for her great help and guidance.

Disclosure statement

No potential conflict of interest was reported by the author.

Funding

The author gratefully acknowledges the funding for a post-doc scholarship visit to the USA offered as a grant by the Egyptian Mission Department, Cairo, Egypt.

References

[1] Heydari A, Pessarakli M. A Review on biological control of fungal plant pathogens using microbial antagonists. J Biol Sci. 2010;10(4):273–290.

[2] Nakkeeran S, Dilantha Fernando WG, Siddiqui ZA. Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. In: Siddiqui ZA, editor. PGPR: biocontrol and biofertilization. Dordrecht: Springer; 2005. p. 257–296.

[3] Tamm L, Thüirig B, Fliesbusch A, Goltlieb AE, Karavani S, Cohen Y. Elicitors and soil management to induce resistance against fungal plant diseases. NJAS - Wagen J Life Sci. 2011;58:131–137.

[4] Pal KK, McSpadden Gardener B. Biological control of plant pathogens. The Plant Health Instructor. [Internet]. APS Net. c2006 American Phytopathological Society [cited 2014]. Available from: http://www.apsnet.org/edcenter/advanced/topics/Pages/BiologicalControl.aspx

[5] Ji SH, Paul NC, Deng JX, Kim YS, Yun B, Yu SH. Biocontrol Activity of *Bacillus amyloliquefaciens* CNU14001 against Fungal Plant Diseases. Mycobiology. 2013;41:234–242. doi:10.5941/MYCO.2013.41.4.234

[6] Wang AS, Hu P, Hollister E B, Rothlisberger KL, Somerhalley A, Provin TL, Hons FM, Gentry TJ. Impact of Indian mustard (*Brassica juncea*) and flax (*Linum usitatissimum*) seed meal applications on soil carbon, nitrogen, and microbial dynamics. Appl Environ Soil Sci. 2012; 351609. doi:10.1155/2012/351609

[7] Bernard E, Larkin RP, Tavantzis S, Erich MS, Alyokhin A, Gross SD. Rapeseed rotation, compost and biocontrol amendments reduce soilborne diseases and increase tuber yield in organic and conventional potato production systems. Plant Soil. 2014;374:611–627.

[8] Mazzola M, Brown J. Efficacy of brassicaceous seed meal formulations for the control of apple replant disease in conventional and organic production systems. Plant Dis. 2010;94:835–842.

[9] Mazzola, M. Transformation of soil microbial community structure and *Rhizoctonia*-suppressive potential in response to apple roots. Phytopathology. 1999;89:920–927.

[10] King EO, Ward MK, Raney DE. J Lab Clin Med. 1954;44:301–307.

[11] Pérez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C. Response of soil microbial communities to compost amendments. Soil Biol Biochem. 2006;38:460–470.

[12] Chu H, Lin X, Fuji T, Morimoto S, Yagi K, Hu J, Zhang J. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long term fertilizer management. Soil Biol Biochem. 2007;39:2971–2976.
[13] Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol. 2003;69:1800—1809.

[14] Mandic L, Djukić D, Beatovic I, Jovovic Z, Pesakovic M, Stevovic V. Effect of different fertilizers on the microbial activity and productivity of soil under potato cultivation. Afr J Biotechnol. 2011;10(36):6954—6960.

[15] Zaccardelli M, De Nicola F, Villocco D, Scotti R. The development and suppressive activity of soil microbial communities under compost amendment. J Soil Sci Plant Nutr. 2013;13(3):730—742.

[16] Lin XJ, Wang F, Cai HS, Lin RB, He CM, Li QH, Li Y. Effects of different organic fertilizers on soil microbial biomass and peanut yield. In: Gilkes R, Prakongkep N, editors. 19th World Congress of Soil Science, Soil Solutions for a Changing World. Proceedings; Vol 7. 2010 Aug 1; Brisbane, Australia; 2010. Available from: http://www.soilsaustralia.com/index.php?option=com_content&view=article&id=83:19wess&catid=24:downloads.

[17] Cohen MF, Yamasaki H, Mazzola M. Brassica napus seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of Rhizoctonia root rot. Soil Biol Biochem. 2005;37:1215—1227.

[18] Kowalchuk GA, van Os GJ, van Aartrijk J, van Veen JA. Microbial community responses to disease management soil treatments used in flower bulb cultivation. Biol Fert Soils. 2003;37:55—63.

[19] Vallad GE, Cooperband L, Goodman RM. Plant foliar disease suppression mediated by composted forms of paper mill residuals exhibits molecular features of induced resistance. Physiol Mol Plant Pathol. 2003;63:65—77.

[20] Diab H, Hu S, Benson DM. Suppression of Rhizoctonia solani on impatiens by enhanced microbial activity in composted swine waste amended potting mixes. Phytopathology. 2003;93:1115—1123.

[21] Veeken AHM, Blok WJ, Curci F, Coenen GCM, Temorshuizen AJ, Hamelers HVM. Improving quality of composted biowaste to enhance disease suppressiveness of compost-amended, peat based potting mixes. Soil Biol Biochem. 2003;37:2131—2140.

[22] Holton J, Boehm MJ. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu Rev Phytopathol. 1999;37:427—446.

[23] Lockwood JL. Relation of energy stress to behaviour of soil-borne plant pathogens and to disease development. In: Hornby D, editor. Biological control of soilborne plant pathogens. Wallingford (UK): CABI International; 1990. p. 197—214.

[24] Tenuta M, Lazarovits G. Ammonia and nitrous acid from nitrogenous amendments kill the microscerotia of Verticillium dahliae. Phytopathology. 2002;92:255—264.

[25] Pharand B, Carisse O, Benhamou N. Cytological aspects of compost-mediated induced resistance against Fusarium crown and root rot in tomato. Phytopathology. 2002;92:424—438.

[26] Bonanomi G, Antignani V, Pane C, Scala F. Suppression of soil-borne fungal diseases with organic amendments. J Plant Pathol. 2007;89(3):311—324.

[27] Ye SF, Yu JQ, Peng JH, Zheng JH, Zou LY. Incidence of Fusarium wilt in Cucumis sativus L. is promoted by cinamic acid, an autotoxin in root exudates. Plant Soil. 2004;263:143—150.

[28] Mazzola M. Assessment and management of soil microbial community structure for disease suppression. Annu Rev Phytopathol. 2004;42:35—59.

[29] Capote N, Pastrana AM, Aguado A, Sánchez-Torres P. Molecular tools for detection of plant pathogenic fungi and fungicide resistance. In: Cumagun CJR, editor. Plant pathology. 2012. p. 151—202.

[30] Caplan B. Organic gardening. London (UK): Headline Book Publ.; 1992.

[31] Mazzola M, Granatstein DM, Elving DC, Mullinix K. Suppression of specific apple root pathogens by Brassica napus seed meal amendment regardless of glucosinolate content. Phytopathology. 2001;91:673—679.

[32] Brown PD, Morra MJ. Control of soil-borne plant pests using glucosinolate-containing plants. Adv Agron. 1997;61:167—231.

[33] Mazzola M, Cohen MF. Suppression of Rhizoctonia root rot by Streptomyces in Brassica seed meal-amended soil. In: Annual international research conference on methyl bromide alternatives and emissions reductions. Proceedings; 2005 Oct 31. MBAO, Fresno, CA.

[34] Mazzola M, Brown J, Izzo AD, Cohen MF. Mechanism of action and efficacy of seed meal-induced pathogen suppression differ in a Brassicaceae species and time-dependent manner. Ecol Epidemiol. 2007;97(4):1215—1227.

[35] Papavizas GC, Lewis JA. Effect of soil amendments and fungicides on aphanomyces root rot of peas. Phytopathology. 1971;61:215—220.

[36] Delledonne M, Xia Y, Dixon RA, Lamb C. Nitric oxide functions as a signal in plant disease resistance. Nature. 1998;394:585—588.

[37] Durner J, Wendehenne D, Klessig DF. Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA. 1998;95:10328—10333.

[38] Huber DM, Watson RD, Steiner GW. Crop residues, nitrite and nitrogen. Plant and disease. Soil Sci. 1965;100:302—308.

[39] Jousset S, Tabachow RM, Pierce JJ. Soil nitric oxide emissions from nitrification and denitrification. J Environ Eng-ASCE. 2001;127:322—328.