EFFECT OF VOLATILES RELEASED FROM SOIL AMENDED WITH RAPESEED MEAL OR RAPE GREENS GROWTH ON THREE SOILBORNE PATHOGENS OF PEANUT.

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ABSTRACT

Sclerotinia minor, Sclerotium rolfsii and Pythium myriotylum were exposed at 22 ± 2C for several days in a closed chamber to volatile chemicals released from soil amended with rapeseed meal (RSM) or rape greens (RG). The volatiles caused reduction in mycelial growth and sclerotial formation of S. minor and S. rolfsii on poato dextrose agar medium (PDA), whereas growth of P. myriotylum on PDA was not affected. Volatiles released from soil amended with RSM (containing about 36 μM glucosinolates/g meal) at 55.000 ppm caused significant (p=0.05) reduction of at 30% reduction in mycelial growth of S. minor and S. rolfsii. Mycelial growth of both fungi was significantly (p=0.05) reduced by about 50% by volatiles released from soil amended with RG (harvested from plants grown from seed meal containing about 37 μ M glucosinolates/g meal) at 55.000 ppm. These results demonstrate that volatile breakdown products of RSM and RG in soil are inhibitory to growth of both S. minor and S. rolfsii.

INTRODUCTION

Several soilborne diseases limit the growth, productivity, and quality of peanut (*Arachis hypogea* L.) in Oklahoma and other peanut producing areas of the United States. The most damaging of these include Southern blight caused by *Sclerotium rolfsii* Sacc. (3), Sclerotinia blight caused by *Sclerotinia minor* Jagger (16, 21, 22), and pod rot caused in part by *Pythium myriotylum* Drechs. (4). Because these pathogens form survival structures such as sclerotia or thick-walled oospores in soil, the diseases they cause are very difficult to control in fields once they are established.

Chemicals are available for management of these diseases in peanut but chemicals add to production cost (3, 16, 20). Some chemicals do not provide acceptable level of disease control and the potential problems of environmental contamination and pesticide residues in food are of an increasing concern to consumers (10, 14, 17). Therefore, alternative control strategies that combine planting resistant cultivars (2) with the use of soil amendments are of interest to the peanut industry. In Oklahoma, Tamspan 90 and Southwest Runner, which are resistant to sclerotinia blight, are used in problem fields (16). Amending soil with organic matter is a strategy that has been successful for controlling *S. minor* on lettuce and *S. rolfsii* (6, 9, 14, 15). Adamsen *et al.* (1) reported that rapeseed (*Brassica napus* L.) meal has the potential to reduce the viability of microsclerotia of *Cylindrocladium crotalatiae* Bell & Sobers, the causal agent of cylindrocladium black rot of peanut. Control probably was due to the sulfur-containing antimicrobial volatiles released from the breakdown of glucosinolates in the rapeseed meal (1,5). The volatiles include mercaptans, sulfides of various types, and isothiocyanates. Isothiocyanates include methyl isothiocyanate, a breakdown product of metham sodium (5). Amending soil with rape green manure and other Cruciferae members also has been reported to be effective against *Rhizoctonia solani*, *Aphanomyces euteiches*, nematodes, and other soilborne pathogens (7, 17, 18).

Rapeseed meal (RSM) and/or rape greens (RG) not only have the advantage of other organic amendments such as stimulating antagonistic microorganisms against pathogens, but they also release biocidal volatiles from glucosinolates against soilborne pathogens. The objective of this study was to assess the inhibitory activity of volatiles released from soil amended with RSM or RG against *S. minor*, *S. rolfsii*, and *P. myriotylum*. Several reports of this research were presented earlier (11, 12, 13).

MATERIALS AND METHODS

Rapeseed meal, contains about 36 μ M glucosinolates/g meal, was obtained from a commercial source (Calgene Chemical Inc., Springfield, IL). Seeds of cultivar Emerald Rape (Calgene Inc.), produce meal containing about 73 μ M glucosinolates/g meal, were planted at the plant pathology Farm in Stillwater, Oklahoma on September, 1992. The greens or the entire above-ground parts of the plants (RG) were harvested in March 1993, dried on a greenhouse bench at about 28 C, and chopped in a blender. The soil used in these tests was a Menofine sandy loam (pH 6.0) from Fort Cobb, Ok. The soil was pulverized to a fine structure before use. The pathogens used in this study, *S. minor*, *S. rolfsii*, and *P. myriotylum*, were maintained on potato dextrose agar (PDA) at 22±1C.

Growth of *S. minor*, *S. rolfsii*, and *P. myriotylum* was tested in the presence volatiles released from soil amended with RSM at 55.000 ppm. Only *S. minor* and *S. rolfsii* were tested in Volatiles released from soil amended with RG at 55.000. RSM or RG were mixed with air-dried soil at the rate of 55.000 ppm. Six hundred g of amended soil was placed in a pyrex glass baking pan (22x12x8 cm). To this, 200 ml of water was added to induce the microbial activity in the mixture, which was necessary

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for the release of the volatile compounds from the degradation of glucosinolates (19). Baking pans containing 600 g of air-dried soil alone to which 200 ml of water was added were used as controls in each test. Pans were then placed in a sealed plexiglass box (32x16x10cm). After incubation for 2 days at 22+2 C, the test organisms were introduced into the closed chamber system as follows: Tissue culture flasks (75 cm^2, canted neck, phenolic style cap, Corning, NY) each containing 40 ml of PDA were each inoculated with a mycelial plug (0.17 cm^2) of the test organism taken from the periphery of a 2-day old culture. The caps of the culture flasks were loosened before placing the flasks onto the surface of the soil mix in the closed plexiglass boxes, so that the volatiles released could enter flasks. Colony growth area of the test organism was measured using an area meter (Delta-T Devices, Cambridge, England), and the time required for formation of sclerotia was recorded. The percentage inhibition of growth caused by the volatiles was calculated as ((100* (1-Growth area in treatment/Growth area in control)). Two weeks after incubation, number of sclerotia formed was counted for *S. minor* and *S.rolfsii* and the number of sclerotia formed per 1 cm^2 of growth area was calculated. Each test was performed twice, except the test with *P. myriotylum*, which was conducted once. Each plexiglass chamber constituted an experimental unit. Each treatment including the controls had four experimental units (replications).

Growth of *S. minor* was also tested in volatiles released from soil amended with RSM minus glucosinolates (RSM-G) at 55.000 ppm. RSM-G was prepared as follows: 300 g of RSM was mixed with 500 ml of 1 N HCl. The mixture was heated at 100 C for 20 min, cooled to 25 C, and filtered through Whatman # 1 filter paper. The extracted RSM was then washed twice with, 1.000 ml of boiling water and dried in an oven at 70 C overnight. Colony growth of *S. minor* in volatiles released from 600 g of air-dried soil alone to which 200 ml of water was added, and in volatiles from soil amended with RSM at 55.000 ppm, were used as controls. Each treatment had three replicates, and the test was conducted once.

Data of sclerotial formation by *S.minor* and *S. rolfsii* were analyzed using ANOVA, and treatment means were compared by Least Significant Difference. Data of the effect of volatiles released from soil amended with RSM or RG on the growth of *S. minor, S. Rolfsii, and P. myriotylum* were analyzed using ANOVA. Only significant (P=0.05) data are discussed unless stated otherwise. All analysis were conducted with SAS (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Mycelial growth of *S. minor* was inhibited when exposed to volatiles released from soil amended with RSM or RG at 55.000 ppm as compared to that of the control. There was a significant difference (p=0.05) of mycelial growth inhibition of *S. minor* exposed to volatiles released from soil amended RG versus RSM. After 50 hr of incubation, mycelial growth of *S. minor* was reduced by 40% by volatiles generated from soil amended with RSM. As compared to 78% inhibition caused by volatiles generated from soil amended with RG. Fewer sclerotia were formed by *S. minor* in volatiles from soil with RSM than that in volatiles from soil alone, and the fewest sclerotia were formed in volatiles released from soil with RG (Table 1). The sclerotial formation of *S. minor* was also delayed about three days under the influence of volatiles released from soil amended with RSM, and three additional days in volatiles released from soil amended with RG (Table 1).

Volatiles released from soil amended with RSM or RG at 55.000 ppm reduced the mycelial growth and number of sclerotia formed by *S. rolfsii*. There was a significant difference (p=0.05) of mycelial growth inhibition of *S. rolfsii* exposed to volatiles released from soil amended RG versus RSM. After 96 hr of incubation, mycelial growth of *S. rolfsii* was reduced by 48% by volatiles generated from soil amended with RSM as compared to 80% inhibition caused by volatiles generated from soil amended with RG. Fewer sclerotia were formed in volatiles from soil amended with RSM and no sclerotia formed in volatiles from soil amended with RG. Sclerotial formation was delayed 13 days in volatiles from soil amended with RSM (Table 2).

For both *S. minor* and *S. rolfsii*, greater inhibition by volatiles were observed in soil amended with RG than in soil amended with the same amount of RSM. This is probably because of the difference in glucosinolates concentration between the two kinds of amendment used. The RSM contained 36 μ M glucosinolates/g meal, but the cultivar of rape from which the RG was obtained had seed meal containing about 37 μ M glucosinolates/g meal. As the concentration of glucosinolates in greens is similar to that in meal (8,19), the RG probably contained more glucosinolates than the RSM.

No inhibition was observed in the mycelial growth of *P.myriotylum* in volatiles released from soil amended with RSM, where growth of colony at 42 hr of incubation was 55 cm^2 as compared to 50 cm^2 in soil without amendment. This study demonstrated that volatiles from soil amended with RSM inhibited mycelial growth and formation of sclerotia a of *S. minor and S. rolfsii*, but did not inhibit growth of *P.myriotylum*. Volatiles released from RSM-amended soil probably have different activities against different types of microorganisms.

No significant difference was observed between the mycelial growth of *S. minor* in volatiles released from soil alone and soil amended with RSM minus glucosinolates (RSM-G). Growth of the pathogen in

volatiles from soil amended with RSM at 55,000 ppm was significantly different from that in volatiles released from soil alone or in RSM-G amended soil. This indicates that the extraction removed the active ingredients (glucosinolates) that contributed to the release of inhibitory volatiles from soil amended with RSM at 55.000 ppm.

No inhibition was observed when *S. minor* and *S. rolfsii* were exposed to volatiles from air-dried soil amended with RSM at 55.000 ppm without the addition of water to the mix (data not presented). The addition of water to the soil amended with RSM or RG is crucial for the decomposition of the glucosinolates present in RSM or RG and the release of volatiles, as water is needed for the growth and reproduction of RSM or RG-decomposing microbes. Therefore, for potential field application, rape plant residues need to be incorporated in the top 3 cm of soil where most infecting propagules of *S. rolfsii* and *S. minor* exist, and then followed by the application of about 2.5 cm of irrigation water to activate the process of breaking down the glucosinolates, and thus releasing the volatile compounds into the soil environment.

Table 1. The effect of volatiles released from soil amended with rapeseed meal (RSM) or rape greens (RG) on the formation of sclerotia of *S. minor*.

Treatment	DAI*	No. Sclerotia/cm^2**
Unamended soil	5 a	19.5 a
RSM (55.000 ppm)	8 b	13.0 b
RG (55.000 ppm)	11 c	5.7 c

* DAI: Days after inoculation when mature sclerotia of *S. minor* were formed. Values are the means of two tests. Each test had four replicates. Means within column followed by the same letter do not differ significantly ($P<0.05$) according to Fisher's LSD test.

** The values are the mean numbers of sclerotia formed per cm^2 on potato Dextrose Agar. Values are the means of two test. Each test had four replicates. Means within column followed by the same letter do not differ significantly ($P<0.05$) according to Fisher's LSD test.

Table 2. The effect of volatiles released from soil amended with rapeseed meal (RSM) or rape greens (RG) on the formation of sclerotia of *S. rolfsii*.

Treatment	DAI*	No. Sclerotia/cm^2**
Unamended soil	12 a	3.55 a
RSM (55.000 ppm)	25 b	0.23 b
RG (55.000 ppm)	–***	0.0 c

* DAI: Days after inoculation, when mature sclerotia of *S. rolfsii* were formed. Values are the means of two tests. Each test had four replicates. Means with column followed by the same letter do not differ significantly ($P<0.05$) according to Fisher's LSD test.

** The values are the mean numbers of sclerotia formed per cm^2 on potato dextrose agar. Values are the means of two test. Each test had four replicates. Means with column followed by the same letter do not differ significantly ($P<0.05$) according to Fisher's LSD test.

*** No sclerotia were observed in this treatment.

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الملخص العربى

تأثير المواد المتطايرة فى التربة والمتحررة من مطحون بذر الشجلم او أجزاءه الخضرية على نمو ثلاث فطريات من قاطنات التربة والتى تصيب نبات الفول السودانى

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تم تعريض الفطريات
Sclerotinia minor , *Sclerotium rolfsii* , *Pythium myriotylum*
على درجة حرارة ٢٢±٢°م لعدة ايام فى غرف مغلقة – للمركبات الكيماوية المتطايرة فى التربة المحسنة بإضافة مطحون بذور الشجلم أو أجزاء خضرية منه. هذه المواد المتطايرة سببت اختزال فى النمو الميسليومى وتكوين الأجسام الحجرية للفطريات *S. rolfsii* و *S. minor* النامية على بيئة دكستروز آجار البطاطس، بينما فطر *P. myriotylum* لم يتأثر بها.
المواد المتطايرة فى التربة والمتحررة من مطحون بذور الشجلم (يحتوى المطحون ٣٦ ميكرومول جليكوسينولات لكل جرام من المطحون) استخدمت بتركيز ٥٥٠٠٠ ج.ف.م. قد سببت اختزال معنوى (*P=0.05*) يقدر بحوالى ٣٠% من النمو الميسليومى للفطر *S. rolfsii* و *S. minor*.
وكذلك النمو الميسليومى لكلا الفطرين اختزال معنويا (*P=0.05*) بحوالى ٥٠% بسبب المواد المتطايرة فى التربة والمتحررة من اجزاء خضرية للشجلم (محصودة من نباتات شجلم منزرعة لبذور يحتوى مطحونها على حوالى ٧٣ ميكرومول جليكوسينولات لكل جرام من المطحون) وذلك بتركيزات ٥٥٠٠٠ ج.ف.م.
النتائج توضح ان هذه المواد المتطايرة من كل من مطحون البذرة والأجزاء الخضرية للشجلم والمتطايرة فى التربة تسبب تثبيط لنمو الفطرين *S. rolfsii* و *S. minor*.