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Annals of Biological Research, 2013, 4 (9):63-66 (http://scholarsresearchlibrary.com/archive.html)



Activity of Volatile Toxins of *Brassica* Residues on Stem and Pod Rot Disease of Groundnut caused by *S. rolfsii* under Green House Conditions

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ABSTRACT

Stem and pod rot of groundnut caused by the pathogen Sclerotium rolfsii, which has a wide host range and it affects the yield losses up to 30-40% in groundnut in India. The persistence of infection caused by sclerotia of S. rolfsii inciting disease stem and pod rot of groundnut following the incorporation of mustard (Brassica nigra) plant parts into soil was examined through an experiment under green house conditions. Incorporation of the mustard plant parts into the soil reduced the infectivity of S. rolfsii at every date at which inoculum was added to soil over the 13 day period of assessment. The effect of adding the mustard plant parts significantly reduced the disease incidence of S. rolfsii. Initially the disease incidence was minimum (37.33%) at 0 day application of S. rolfsii immediately after incorporation of mustard plant parts. Thereafter the disease incidence increased from 1^{st} day of application of pathogen to 5^{th} day (46.66%, 54.00% and 65.33%), respectively. Later from 7^{th} day onwards the disease incidence reduced from 63.66% to 52.33% up to the 13^{th} day.

Keywords: S. rolfsii, mustard, glucosinolates, isothiocynates, groundnut.

INTRODUCTION

Brassica-cover crops are increasingly used as catch crops and/ or green manure crops with in rotations to provide a number of agronomic benefits like control of nitrogen leaching, increased soil organic matter and improved soil structure. They also have the potential to suppress a range of soil borne crop pests and diseases [1, 2]. *Brassica* species contain glucosinolates (GSL), which, upon tissue disruption, are hydrolyzed in the presence of water by an endogenous myrosinase enzyme into numerous compounds, notably toxic isothiocynates (ITC). The detrimental effect of pure ITC to certain fungi has long been known and the potential of *Brassica* crops to control soil borne pests and pathogens mainly attributed to these compounds. This process termed as "bio-fumigation" [3], is increasing interest as it is viewed as an alternative to the use of traditional inorganic soil fumigants in the control of soil pathogens. Brown and Morra [4] reviewed the factors contributing to biofumigation efficacy (efficiency of ITC release, susceptibility of the target species, soil moisture content, etc.) and suggested that, as the life time of GSL products in the environment was shown to be short [5, 6], "a short residue time places limits on achieving effective control and may contribute to the variability observed in the suppression of soil-borne plant pathogens.

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Groundnut (*Arachis hypogea* L.) is cultivated around the world in tropical, sub-tropical and warm temperate climates. The total area under groundnut in India is 6.70 m. ha annually with a total production of 7.16 million tones. Andhra Pradesh ranks first with an area of 1.76 m. ha and annual production of 0.95 million tones. Losses due to seed and seedling diseases particularly stem and pod rot of groundnut caused by *S. rolfsii* have been reported to an extent of 30-50 per cent in individual plots [7]. The aim of this study was to determine the persistence of action of *B. nigra* residues in the soil for the disease control. This was achieved by linking the time at which inoculum was introduced into the soil following the incorporation of *B. nigra* residues to the disease expression of stem rot of groundnut.

MATERIALS AND METHODS

Cultivation of mustard plants

The locally available mustard (*Brassica juncea*) was used for green house experiment. Single mustard plant was raised for each pot. Each earthen pot of 27 cm height and 13 cm radius was filled with 4 kg of potting mixer consisting three parts of red soil and one part of black soil and sufficient amount of urea, single super phosphate (SSP), murate of potash (MOP), farm yard manure (FYM) and vermicompost. The experiment was maintained in completely randomized design with three replications per each treatment.

Preparation of mustard green manure

Sixty day old mustard plants were harvested along with the stem, leaves, flowers, and roots, chopped into small pieces and incorporated into the soil immediately to a depth of 15 cm from the top of the pot [8]. A little quantity of water was added to hydrolyse the glucosinolates and covered with polythene sheet for 15 days. The quantity of mustard plant parts incorporated into the soil is equivalent to 200 g fresh weight per pot. Control pots were maintained without mustard incorporation.

Soil infestation

According to the literature, the life of ITC and GSL in soil was shown to 6 and 8 days, respectively, at the most [8]. As the purpose of this study was to determine the time when the effect of ITC would shut down, it was decided to work on a fine time scale of 13 days. The fungal propagules were incorporated into the soil at different times (0, 1, 3, 5, 7, 9, 11 and 13 days) after the incorporation of mustard plant parts. At each time, ten sclerotia of the fungus *S. rolfsii* were placed into the soil at a depth of 1cm from the top of the soil. Eight treatments and five replications were maintained in a completely randomized block design (Table I).

Table I. The treatments and combinations

S. No	Treatments
1	T_1 -Pathogen application on the same day of mustard incorporation (0 day)
2	T ₂ -Pathogen application on 1 st day after mustard incorporation
3	T ₃ -Pathogen application on 3 rd day after mustard incorporation
4	T ₄ -Pathogen application on 5 th day after mustard incorporation
5	T ₅ -Pathogen application on 7 th day after mustard incorporation
6	T ₆ -Pathogen application on 9 th day after mustard incorporation
7	T ₇ -Pathogen application on 11 th day after mustard incorporation
8	T ₈ -Pathogen application on 13 th day after mustard incorporation

Fifteen days after incorporation of mustard residues a seed of groundnut was sown 1cm away from the sclerotial bodies. Fertilizers were not applied before and after 15 days of incorporation of mustard plant parts in the pots. The infectivity of *S. rolfsii* was estimated based on the number of groundnut plants infected. The seedlings were examined visually, for the presence of dark-brown lesions and white mycelia growth and the disease incidence was calculated by using the following formula as described by Mayee and Datar [9].

RESULTS AND DISCUSSION

The experiment was conducted in glass house, Department of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad under artificially inoculated conditions to evaluate the persistence of mustard crop residues in the soil in reducing infection caused by sclerotia of *S. rolfsii* and the results are presented in table II.

Treatment	Plant height(cm)	PDI
T_1 - Pathogen application on the same day of mustard incorporation (0 day)	22.00	37.33
		(37.64)*
T ₂ - Pathogen application on 1 st day after mustard incorporation	19.16	46.66
		(43.07)
T ₃ - Pathogen application on 3 rd day after mustard incorporation	18.33	54.00
		(47.28)
T ₄ - Pathogen application on 5 th day after mustard incorporation	15.50	65.33
		(53.91)
T ₅ - Pathogen application on 7 th day after mustard incorporation	15.76	63.66
		(52.92)
T ₆ - Pathogen application on 9 th day after mustard incorporation	16.00	60.33
		(50.94)
T ₇ - Pathogen application on 11 th day after mustard incorporation	16.66	57.33
		(49.20)
T ₈ - Pathogen application on 13 th day after mustard incorporation	22.33	52.33
		(46.32)
S. Em ±	1.41	1.74
		(1.01)
CD at 5%	4.27	5.28
		(3.07)

Table II. Effect of volatile compounds of Brassica residues at different dates of soil infestation of S. rolfsii under green house

*Figures in the parentheses indicate angular transformed value

The incorporation of the mustard plant parts into the soil reduced the infectivity of *S. rolfsii* at every date at which inoculum was added to soil over the 13 day period of assessment. The effect of adding the mustard plant parts significantly reduced the disease incidence of *S. rolfsii*. Initially the disease incidence was minimum (37.33%) at 0 day application of *S. rolfsii* immediately after incorporation of mustard plant parts. Thereafter the disease incidence increased from 1st day of application of pathogen to 5th day (46.66%, 54%, 65.33%) respectively. Later from 7th day onwards the disease incidence was reduced from 63.66% to 52.33% up to the 13th day.

Similar trend was observed with regard to plant height of groundnut when brassica plant parts were incorporated into the soil. The plant height was reduced from 0 day to 5th day (22cm to 15.5cm) and from 7th day there was increase in plant height of groundnut from 15.76 to 22.33cm though not much variation was found. It was also observed that in the inoculated control the groundnut did not germinate until 3^{rd} day of application. From 5^{th} day onwards groundnut seeds germinated though with poor vigour and the height of the plant was only 5cm and later the plants wilted. Results of Motisi et al. (2009) also indicated that incorporation of above ground parts alone (AP) or the above-ground parts combined with the below ground parts (AP+BP) reduced the infectivity of inoculums of both Rhizoctonia solani and Gaeumannomyces graminis var. graminis tritici at every date at which inoculums was added to soil over the 13-day period of assessment. However, the persistence of ITC in soil after the grinding and incorporation of *Brassica* residues was shown to be short and according to previous studies, no longer than 3 days has been reported by Gardiner et al.[11], Morra and Kirkegaard [4] to 6 days [12]. In the present investigation the mustard plant parts were mixed with the soil in all the pots at a time and the pathogen S. rolfsii was added from 0 day onwards until the 13th day. Later the groundnut seeds were sown 1 cm away from S. rolfsii on the 15 day at a time in all the pots. It is clearly seen that by the time the mustard plant parts started producing the volatiles the sclerotia of S. rolfsii also took time to germinate and proliferate in the soil from 0 to 5 days. But the mustard volatiles did have an effect on germination of groundnut and the plant height. In spite of the pathogen the groundnut seed could germinate and recorded a significant plant height. From the 7th day the pathogen was suppressed and the groundnut plant height also increased. Present investigation also showed that the persistence of brassica residues was significantly shorter than the persistence of residue action. However these results provide valuable insight into the relative efficacy of control afforded by the brassica residues considering the small amount of material added and the toxin released into the soil and the suppressive capacity of the residues is remarkable. On contrary, a recent study of Mazzola et al. [13] demonstrated that the suppression of R. solani AG-5 by Brassica juncea seed meal amendments was associated with the release of allylisothiocynate in the first day. This supports the present results on the temporal changes in the efficacy of disease control by B. juncea residues. The following explanation for the trends observed in the present experimentation is proposed that the describing changes in the control of S. rolfsii over time for mustard residues were the result of an initial decrease in efficacy (during the first 5 days) resulting from the fast disappearance of the toxins released by the residues.

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