

Essential Oil of Mustard to Control *Rhizoctonia solani* Causing Seedling Damping off and Seedling Blight in Nursery

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ABSTRACT

The essential oil extracted from mustard (*Brassica rapa*) seeds was evaluated for its effect on suppression of *Rhizoctonia solani* growth *in vitro*, and in field soils, for reducing saprophytic substrate colonization and seedling damping off and blight using snap beans as indicator plant, the *in vitro* growth was completely inhibited at a concentration of 50 $\mu\text{l/l}$. The saprophytic substrate colonization in soils 24 h after treatment was drastically reduced to 45% at 150 $\mu\text{l/kg}$ soil concentration, in contrast to 100% colonization at concentrations of 0, 50, or 75 $\mu\text{l/kg}$. This recovery rate gradually declined to 6% and 60%, respectively, in nine days.

A control of pre and post-emergence seedling damping off and blight in common beans (*Phaseolus vulgaris*), without any apparent phytotoxic effect was achieved by irrigating *R. solani* infested soils with water containing the emulsified essential oil to provide 150 $\mu\text{l/l}$ soil volume ten days prior to planting, gave over 95%. The effect of the mustard essential oil was not influenced by the physical soil texture, and it appears to be a good substitute for methyl bromide fumigation in nurseries for seedling production.

Additional keywords: allyl isothiocyanate, flavoring agent, soil treatment, nursery.

RESUMO

Utilização de óleo essencial de mostarda no controle de tombamento e requeima causadas por *Rhizoctonia solani* em viveiro

Foi avaliado o efeito do óleo essencial extraído de sementes de mostarda (*Brassica rapa*) na supressão do crescimento *in vitro* de *Rhizoctonia solani*, na redução da colonização saprofítica no solo, e no tombamento e requeima de plântulas, utilizando feijão-vagem (*Phaseolus vulgaris*) como planta indicadora. O crescimento *in vitro* de *R. solani* foi completamente inibido na concentração de 50 $\mu\text{l/l}$. A colonização saprofítica do substrato foi drasticamente reduzida para

45% na concentração de 150 $\mu\text{l/kg}$, 24 h após o tratamento. Nas concentrações de 0, 50 e 75 $\mu\text{l/kg}$ de solo houve 100% de colonização do substrato. Esta taxa de recuperação diminuiu gradualmente para 6% e 60%, respectivamente, em nove dias. A irrigação de solos infestados por *R. solani* com água contendo o óleo essencial emulsificado para fornecer 150 $\mu\text{l/l}$ de solo, dez dias antes do plantio, resultou em 95% de controle do tombamento e requeima em mudas de feijão-vagem, sem qualquer efeito aparente de fitotoxidez. O efeito do óleo essencial não foi influenciado pela textura do solo e parece ser um bom substituto para o fumigante brometo de metila em viveiros.

The losses caused by the *Rhizoctonia solani* Kühn in seedling production of transplant perennial [eucalyptus (*Eucalyptus* spp.), coffee (*Coffea arabica* L.), etc.] or vegetable crops [cabbage (*Brassica oleracea* L.), tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum* spp.), etc.] are high in nurseries as well as in the field when asymptotically infected seedlings are transplanted. Generally, *R. solani* is eradicated from the seedling substrate by fumigation with methyl bromide. This highly toxic fumigant that also damages the ozone layer is being gradually prohibited throughout the world. In Brazil its use has been restricted to specific purposes; beginning December 2006 it will be prohibited for use in nurseries (MAPA, 2002). However, there is no efficient substitute available for nursery use at this time.

Several studies have shown that amending the soil with plants of *Brassica* spp. can reduce the disease incidence or inoculum level of *Macrophomina phaseolina* (Tassi) Goid., *Pythium ultimum* Trow., *Fusarium oxysporum* Schl. f. sp.

cumini Patel, Prasad & Mathur and *R. solani* (Charron & Sams 1999, Mawar & Lodha, 2002, Lodha & Sharma, 2002). Seed treatment with *Brassica* spp. seed meal to control *R. solani* in cabbage has been reported (Chung *et al.*, 2002). The mechanism involved in the disease control or suppression of a pathogen is considered to be the production of allyl isothiocyanate (AITC) by the tissues of *Brassica* spp. (Olivier *et al.*, 1999). Antimicrobial activity of AITC is well documented in the literature, and it can kill fungal propagules by vapor action, thus acting as a fumigant (Lin *et al.*, 2000, Nielsen & Rios, 2000; Mari *et al.*, 2002). This volatile compound is produced in an aqueous medium through enzymatic hydrolysis of sinigrin, which is the predominant glucosinolate in the tissues of *Brassica* spp. Among the *Brassica* spp. the highest concentrations of AITC are found in some mustered (*Brassica juncea* L.), horseradish [*Armoracia* (Gaertn.) Mey. & Scherb] and wasabi (*Wasabia japonica* Matsum) species, with considerable variation among

the cultivars of the same species (Olivier *et al.*, 1999).

Although soil amendments with fresh mustard leaves or seed meal have shown promising results for controlling soil born pathogens, results can be inconsistent due to variations in AITC content. The use of fresh plant materials is unsuitable in the nursery because there is no practical method for standardizing their processing and the materials are not readily available. Also, there is no information regarding the fate of AITC during storage and the factors that influence its production in amended soil. These restraints can lead to inconsistency of disease control. A ready to use, standardized product is more desirable in nurseries to ensure consistent control of soil borne *R. solani*. The AITC rich essential oil can be extracted from seeds or other plant tissues. This essential oil is widely used as a flavoring agent by the food industry and is classified as generally regarded as safe (GRAS) by the Food and Drug Administration of the United States. Therefore, the following exploratory study was done to determine the feasibility of using the mustard essential oil for soil treatment to control *R. solani* inoculum in a substrate for seedling production.

The essential oil was extracted from mustard seeds using the following procedure. Five kilogram of mustard seeds were ground to pass through a 0.5 mm screen and soaked in 10 l of water. The mixture was allowed to stand for 4 h at room temperature for hydrolysis of glucosinolates and then hydrodistilled using a stationary distillator at 100 °C and air flow of 5 l/min. The distillate containing water and the essential oil was collected into several separatory funnels, and allowed to stand for 2 h to separate the oil from the water phase. The water phase was discarded and the collected essential oil was dehydrated by passing through a 5-cm column of anhydride sodium sulfate. It was then emulsified with 10% Tween® 20 and refrigerated until use.

The *R. solani* isolate used in this study was obtained from snap bean (*Phaseolus vulgaris* L.) seedlings collected from a commercial vegetable field having a history of extensive damage caused by the fungus. After isolation, the pathogenicity of the isolate was tested by placing a three-day old potato-dextrose agar (PDA) culture disc on the soil surface close to the collar region of snap bean seedlings growing in pots containing methyl bromide treated soil. The collar rot symptoms developed within four days and the fungus was reisolated from the diseased portion of the seedlings, and the stock culture was stored over silica gel for further use. All new cultures of the fungus were started using this stock culture, avoiding repeated sub-culturing on artificial culture media. The *R. solani* inoculum for soil infestation was produced on autoclaved snap bean pods, dried and ground to 1 mm mesh (Dhingra & Sinclair, 1995).

To determine the effect of the mustard essential oil on *in vitro* growth of *R. solani*, the fungus was cultivated on PDA containing the essential oil at concentrations of 0, 10, 25, 50, 75 or 150 µl/l. The emulsified oil was thoroughly mixed with 60-ml cool molten PDA, and the mixture was poured into five 9-cm culture plates. The medium in each

culture plate was seeded with a 5-mm culture disc obtained from the advancing margin of *R. solani* colony growing on water agar, and incubated at 25 °C. The fungal growth was observed daily for 15 days, although in the control plates the fungus had reached the margins in four days.

To study the effect of the essential oil on the saprophytic activity of *R. solani* in soil, three soils of different textures were used. The soils were characterized as sandy-loam (65% sand, 22% silt, and 13% clay, water holding capacity (WHC) 32%), silty-clay loam (25% sand, 44% silt 31% clay, WHC 54%), and sandy clay loam (69% sand, 7% silt, 24% clay, WHC 42%). The soils were collected from three different fields of vegetable production, with histories of *R. solani* damage to vegetable crops. The soils were air-dried, sieved through 1.5 mm sieve, and the inoculum of *R. solani* was augmented by mixing the dry inoculum (5 g/kg soil) in a twin arm mixer.

The soils were treated with the essential oil at concentrations of 0, 50, 75, and 150 µl/kg dry soil. A 250 g portion of each soil was placed in an appropriate plastic bowl, emulsified essential oil mixed with enough water to moisten the soil to 50% WHC was added and thoroughly blended with an electric blender. The saprophytic activity was determined through colonization of snap bean petiole segments. Petioles were collected from lower leaves of six-week old snap bean plants, and were cut into 1-cm long segments, boiled for 2 min, blot-dried, and 20 of them were mixed with the soil. The mixture was then tap-packed in 250-ml beakers, which were then covered with a polyethylene film and incubated at 25 °C. The petiole segments were recovered from soil, by wet sieving, one, three, six, and nine days after incubation. There was a separate beaker for each sampling date. The recovered segments were washed under running tap water with gentle scrubbing with a soft brush to remove the closely adhered soil particles, blot-dried, surface disinfested by dipping in 70% ethanol for 1 min and then plated on the selective medium (Ko & Hora, 1971, 1995). The segments yielding *R. solani* were counted after an incubation of three days at 25 °C.

The capacity of the essential oil of mustard to control *R. solani* seedling blight and damping off was studied using sandy-loam and silty-clay-loam soil with augmented inoculum as described earlier. The soil was distributed into perforated plastic trays (50 L x 25 W x 30 D cm) to a depth of 25 cm and irrigated to full WHC and 50 snap bean seeds were planted in each tray to determine the uniformity of disease incidence. The seedlings with *R. solani* symptoms were counted at the fully expanded primary leaf stage and then discarded.

The soil in each tray was then treated with the essential oil at the rate of 150 µl/l soil volume by irrigating with 2 l of water containing the required amount of the essential oil. Soil irrigated with water only served as control. Ten days after the treatment 30 snap bean seeds were planted in each tray. No further treatment was given except irrigation as required. The number of emerged seedlings and seedlings with *R. solani* symptoms was counted 25 days after seedling emergence. Isolations were made from five to ten, randomly

picked diseased seedlings to confirm the identity of the causal agent.

All the experiments were done in a completely randomized block design with three replications and repeated twice. After analysis of variance, the means were compared by least significant difference (LSD).

The colonies of *R. solani* in control plates reached the plate margins (9-cm) in four days after seeding, while no growth was observed during the 15 days on the medium containing 50 µl/l or higher concentrations of the essential oil. In culture plates containing 10 or 25 µl/l, the growth was visible only after two and four days and the colony diameter reached only 5 and 3 cm after 15 days, respectively.

In the saprophytic activity test in soils, the proportion of bean petioles yielding *R. solani* did not differ ($p > 0.05$) among the soil types, independent of the treatment. Therefore the data from the three soils were pooled and reanalyzed using nine replications to determine the effect of the essential oil concentration on the saprophytic activity of *R. solani*. The proportion of bean petiole segments yielding *R. solani* was highest 24 h after treatment in all the treatments and started to decline thereafter. In non-treated soils or soils treated with 50 or 75 µl/kg of the essential oil, the initial *R. solani* recovery rate of 100% declined to an average of 85, 72, and 60%, three, six, and nine days after the treatment, without significant difference among the treatments and the control. But in soils treated with 150 µl/kg of the essential oil, the *R. solani* recovery rate was only 45, 27, 11, and 6% in the corresponding sampling periods.

The total incidence of pre- and post-emergence seedling damping off and blight in snap beans, in all soil types, averaged 5% compared to 92% in non-treated soils without significant difference among soils. There was no apparent phytotoxicity in seedlings.

In the second run of the entire study, tendency and biological significance of the treatment were similar to the first run, except for small quantitative differences in the soil tests.

The high control of snap bean seedling damping off and blight resulting from the reduction of saprophytic activity of *R. solani* as found in this initial exploratory study and the other reports (Gilardi *et al.*, 2002, Harvey *et al.*, 2002) strongly indicate, that the essential oil of mustard may successfully substitute methyl bromide soil fumigation to control some plant pathogens in nurseries. An *in vitro* toxic effect of AITC (the major component of this essential oil) on several fungi has also been reported (Lin *et al.*, 2000; Nielson & Rios, 2000). The amount of the essential oil required to completely inhibit *in vitro* growth of *R. solani* and saprophytic substrate colonization differed considerably, which suggests that *in vitro* testing can be misleading for determining the most effective concentration for soil treatment. The *in vitro* growth of *R. solani* was totally inhibited at low concentrations of 50 µl/l and drastically reduced even at lower concentrations of 10 or 25 µl/l. Such magnitude of *in vitro* growth reduction suggests the high toxicity of mustard essential oil towards *R.*

solani. But, the saprophytic growth test showed completely different results where the proportion of saprophytically colonized substrate units did not differ from the control. Even at a concentration three fold higher than the *in vitro* inhibitory concentration, 45% of the substrates units yielded *R. solani*, although this number declined to about 6% in nine days. These results suggest that although the fungal activity at this concentration was significantly reduced, the action was neither rapid nor as lethal as shown by the *in vitro* studies. The continuous decrease in fungal recovery from the substrates clearly shows fungal mortality even in the colonized substrates. On the other hand, evaluation of the real potential of mustard essential oil for soil treatment could be highly influenced by the technique used for the assay. Thus, for pathogens like *R. solani*, which have active saprophytic growth in soil, the estimation of saprophytism may provide a more realistic picture than the *in vitro* assays.

Rhizoctonia solani must grow saprophytically through soil prior to infecting seed or seedlings and there is a very close relationship between the two parameters (Baker & Martinson, 1970). Since *R. solani* saprophytically colonized the substrate in treated soils, it was assumed that disease control would be inefficient if the host was planted shortly after soil treatment because the fungus would have made saprophytic growth, essential for seedling infection. The ten-day wait period between the treatment and planting may explain the drastic disease control.

The data from this initial study, as well as those of others, strongly suggest that mustard essential oil has the capacity to control some of the soil born pathogens in the nursery independent of the soil texture, thus warranting detailed and extensive research to develop it into an effective soil treatment of fungicides for substituting methyl-bromide.

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