Indian mustard (*Brassica juncea*): A potential fungicide for control of early blight (*Alternaria solani*) of potato

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Abstract

Early blight of potato in India causes yield losses from 10-90% in mild to severe infestation. Different combinations of *Brassica juncea* oil meal were compared for their volatile compound in hydrated form that was fungicidal to *Alternaria solani.* Indian potato (*Solanum tuberosum*) variety Kufri Jyoti was selected as an indicator crop for the treatment of *Alternaria solani.* Among the samples tested the volatile substrate in the oil meal of mustard (*Brassica juncea* (L.) *Coss. & Czern.*) with 50% carrier showed the strongest fungicidal effect. Allylisothiocyanate was the predominant fungicidal compound detected in the hydrated oil meal of all treatments. Among 6 carrier combinations tested for seed treatment with ground mustard oil meal, carrier-5A (C-5A) containing Indian charcoal trema (50%), calcium carbonate (1%), barley flour (30%), rice bran (10%) and gum arabic (9%) was the most effective for the seed treatment at 1:1 oil meal and carrier ratio. Seed potato treated with a mixture of mustard oil meal and Carrier-5A (1:1) at 7% (w/w) resulted in a significant reduction in incidence of *Alternaria solani* with no harmful effects to the germination of treated seeds. Effective control of *Alternaria solani* by the seeds treatment with Carrier-5A mixture was also obtained in a large scale experiment conducted on a field level. This study suggests that the mustard oil meal and carrier–5A (C-5A) mixture has potential for use in the commercial seed treatment for early blight of potato.

Key words: Early blight, *Alternaria solani*, *Solanum tuberosum* ground mustard meal, volatile compound, allylisothiocyanate, *Brassica* spp., carrier mixture

Introduction

Early blight has been a problem for potato (*Solanum tuberosum*) growers since the 1893 (Gary et al., 1997). The disease is caused by the fungus *Alternaria solani*, which can also attack tomato and some weeds of the solanaceous family. Under highly congenial conditions early blight appears in epiphytotic form and kills the entire foliage within a few days. Potato vines that remain wet for long periods create a microenvironment conducive to early blight *Alternaria solani* (Curwen, 1993). Yield is reduced by 30-50% from defoliation and fruit rot and early season outbreaks may result in reduced fruit production and in severe infestation can damage the entire field (IAS, 1998). No fungicides are currently labeled for application to tubers to reduce tuber infections after harvesting (Gary et al., 1997). The chemical fungicides are developing resistance among pathogens and degrading the soil and aqua ecosystem. Therefore, an alternative for effective control is required without depleting the ecosystem. One such promising crop is Indian mustard (*Brassica juncea*) containing bioactive compound allylisothiocyanate (AITC) that can be utilized for effective control of *Alternaria solani.*

Mustard seed meal containing glucosinolate as a soil amendment controls plant pests to a great extent (J. Brown, M.J. Morra, 2002). Dandurand et al. (2000) used *B. napus* cv. "Dwarf Essex" meal in laboratory experiments to control *Sclerotinia sclerotiorum* and *A. euteiches*. A 100% reduction in carpogenic germination and a 33% reduction of myceliogenic germination of *S. sclerotiorum* occurred in soil amended with Dwarf Essex meal. Soil amended with *Brassica* residues as green manure (Gamliel and Stapelton, 1993) or *Brassica* seed meal (Smolinska et al., 1997) induced suppression to plant fungal pathogens. The suppressive effects are generally attributed to biocidal compounds such as glucosinolates released from the crop residues or seed meal after hydrolysis (Angus et al., 1994; Smolinska et al., 1997). These hydrolytic compounds have antimicrobial, fungicidal, and insecticidal properties, with isothiocyanates (ITC) as the most toxic compounds (Banks et al., 1986; Fenwick et al., 1983). Therefore, the anti-fungal volatile substance from *Brassica* crops may have potential of replacing synthetic chemicals for control of soilborne plant pathogens.

Materials and Methods

Seed, meal and carriers: Mustard seed meal of *Brassica juncea* was obtained from different edible oil manufacturers from the local market. Certified seed potatoes were also purchased from a local seed supplier (variety Kufri Jyoti). Carrier materials were also obtained from locally available manufacturers/suppliers. The different carriers were nursery moss, cellulose, hemicellulose, magnesium oxide, oyster shell powder Indian charcoal trema, barley flour, rice bran, calcium carbonate, wheat bran, Silicone and dioxide. The binder used was gum arabic. All the carrier ingredients were made in powder form in a grinder for their further use. The composition of the finally selected carrier (C-5A) was Indian charcoal trema (50%), calcium carbonate (1%), barley flour (30%), rice bran (10%) and gum arabic (9%).

Analysis of Volatile compounds in seed meal: The mustard oil meal (15gms) was ground in a Black & Decker grinder for 2 minutes. From the ground material 1gm was taken in a glass flat-bottomed flask and 70% ethanol was added in the ratio of 1:4. This mixture was kept on a magnetic stirrer for 1 minute and then boiled on water bath for 5 minutes then vigorously shaken for 2 minutes and centrifuged at 1000×g for 5 minutes. Then 20µl of supernatant was separated and injected in a Shimadzu-10Avp-Class High Performance Liquid Chromatography (HPLC) system with LC-18-5µm column at wave length of UV-226nm (Graser et al.,2000).

Infestation of culture media and screening of Brassica meals for A. solani inhibition: Alternaria solani isolated from an infected potato plant showing early blight symptoms was used in this study and was conformed after primary culture. Also mother culture of *Alternaria solani* was obtained from National Botanical Research Institute (NBRI), Lucknow. Agar blocks (0.5 cm in diameter) containing mycelial mats of the said pathogen was removed from 3-day-old cultures grown on potato dextrose agar (PDA) maintained at 28°C was used for infestation. Fresh oil meals of *Brassica juncea* were ground in a grinder (Black & Decker, UK) for about 2 minutes. Different concentrations of oil meal like 0.1, 0.2, 0.3, 0.4 and 0.5gm of the ground oil meal was mixed with sterile distilled water at a ratio 1:1 (w/v) in Petri dish (9 cm in diameter). Petri dishes containing PDA amended with 150ppm streptomycin sulfate were placed upside down to prevent any bacterial development, might be associated with oil meal (J.W. Huang et al., 2002). The bottom of each plate was inoculated with *Alternaria solani* by placing an agar block with mycelia (0.5 cm in diameter) obtained from 3-day-old PDA cultures.

Selection of binders/carriers for seed treatment: Six carriers, including C-1A, C-2A, C-3A, C-4A, C-5A and C-6A were used in this study. The selection of carrier was based on its holding capacity of allylisothiocyanate for maximum time. To test each carriers holding capacity, mustard oil meal was mixed with each of the carriers at 1:1 ratio (w/w). Fresh soil was taken and autoclaved and filled in disposable cups (100gms). The carrier and oil meal mixture was added in each cup at the rate of 10% of soil weight and kept for 15 days having 20-replicates of each carrier. Upto fifteen days each carrier cup was harvested and analyzed for residual allylisothiocyanate retained in the soil (substrate) by HPLC-system described previously. The carriers were also analyzed for their efficacy for germination percentage and *Alternaria solani* inhibition along with mustard oil meal at the same ratio of 1:1 (w/w).

Effect of mustard oil meal and carrier C-5A on seed germination and early blight of potato: Ground mustard oil meal was mixed with carrier C-5A at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% (w/w). Each mixture was used to coat seed potatoes using gum-arabic as a coating (binder) medium, the potatoes were presoaked in a solution of 0.1% $AgNO_3$for 30 seconds for surface sterilization. Potatoes treated with silver nitrate were used as controls. The treated and untreated seeds were sown in soil media artificially infested with *Alternaria solani*. There were 20 replicates for each treatment. The germination percentage, disease incidence percentage and yield was determined. The optimum applied percentage of carrier C-5A and oil meal mixture with respect to potato for seed treatment was also analyzed. The percentages analyzed were 1, 2, 5, 7, 10 and 15% (percentage of seed treatment mixture with respect to potato weight) to determine the percentage of treatment mixture for commercial utilization.

Statistical analysis: Data on mycelial growth, disease incidence, seed germination, carrier efficacy etc. were analyzed by using analysis of variance (ANOVA).

Results

Inhibition of Alternaria solani by volatile compound of Brassica oil meal: The effect of volatile compound from hydrated *Brassica* oil meal on mycelial growth of *Alternaria solani* varied with the quantity of ground mustard oil meal used (Table 2). From the 10 ground *Brassica* oil meal concentrations tested, the most effective volatile compound was released by 0.2gm and above treatments, which completely inhibited mycelial growth of *Alternaria solani* (Table 1). When the inoculum of *A. solani* exposed to the said treatments for 3 days at 28°C was transferred to a fresh PDA and incubated for further 3 days, there were no signs of mycelial growth. Volatile compound of ground *Brassica* oil meal (allylisothiocyanate) released by 0.3gm was approximately 1000ppm. The effect of pure allylisothiocyanate on the growth of *Alternaria solani* was also analyzed. Mycelial growth of *Alternaria solani* was reduced when the cultures were exposed to allylisothiocyanate at the concentrations of 100–250ppm and was completely suppressed at and above concentration of 500ppm.

Table-1: *In vitro* screening of oil meal for *Alternaria solani* inhibition

S.No.	Oil meal [a] concentrations	% inhibition [b] (*Alternaria solani*)	Residual [c] AITC (ppm)
1.	0.1g	72%	54 *(1.9)*
2.	0.2g	100%	61 *(3.9)*
3.	0.3g	100%	65 *(6.4)*
4.	0.4g	100%	89 *(3.9)*
5.	0.5g	100%	111*(4.6)*
6.	Control (water)	0%	--------
7.	Control (Streptomycin)	0%	---------

[a] Oil meal concentrations per petri dish hydrolyzed with 1:1 sterilized water, and control treatments of both water and 150ppm streptomycin sulphate.
[b] Percentage inhibition of *Alternaria solani* significant at *(P*=0.05) average of 10 replicates.
[c] Residual concentration of allylisothiocyanate in the petri dishes, results are based on 10 replicates of each sample.

Carrier/binder material selection for seed treatment: The carrier C-5A was selected because of its maximum holding capacity of allylisothiocyanate (13days at 28° ± 2°C) as compared to others where most of the carriers could not cross 10 days holding capacity. The carriers used for mixing with mustard oil meal at a 1:1 ratio (w/w) after surface sterilization of seed potatoes, had significant effects ($P<0.05$) on seed germination and disease inhibition. All carriers showed no adverse effects on seed germination with a germination rate of 100% compared with the controls. The carrier C-5A mixed with the ground mustard oil meal caused a significant reduction in incidence of early blight of potato. Among the treatments, the residual concentration of allylisothiocyanate was the highest (56.4 ppm) in the substrate mixed with the ground mustard oil meal and carrier C-5A mixture on 13^{th} day analysis.

Analysis of ratio of carrier and oil meal mixture for seed treatment: The ratio of the mustard oil meal and the carrier C-5A used for treating seed potatoes affected seed germination and *Alternaria solani* incidence of potato when carrier mixture is used more than 70%. Compared with the controls, seed germination was not inhibited when the amount of the mustard oil meal in the mixture was reduced to 50% or lower. Very less seed germination was observed when the seeds were treated with 100% of the ground mustard oil meal. Although the ground mustard oil meal mixed with the carrier C-5A from 1:1 to 1:9 were all effective in reducing incidence of early blight of potato, the optimum ratio for the control of this disease was oil meal to carrier of 1:1(w/w).

Carrier and oil meal percentage analysis for seed treatment: The carrier C-5A and oil meal mixture in the ratio of 1:1 was further analyzed for better performance percentage. The percentages analyzed were 1, 2 , 5, 7, 10 and 15% with respect to potato weight. From the Table-2, it is obvious that at 7% mixture weight the germination is not effected, disease incidence is reduced and yield is better compared to control. As in the other ratios, either there is less germination or the occurrence of disease is more. Therefore, the oil meal mixture along with carrier C-5A in the ratio of 1:1 at 7% is suitable for commercial utilization for treating early blight of potato (Table-2).

Table-2: Effect of different concentrations/ percentage of treatment mixture on potato

S.No.	Oil meal carrier (%) *	Seed germination (%)*	Disease incidence (%)*	Avg. yield (Kg) * per plant
1.	1	100	59	1.44
2.	2	100	56	1.48
3.	5	98	52	2.26
4.	7	99	36	2.59
5.	10	88	32	1.59
6.	15	82	32	1.48
7.	Control ($AgNo_3$)	100	64	1.42
8.	Control (Water)	100	67	1.40

*Seed germination, disease incidence percentage, and yield average of 20 replicates significant at (P=0.05)

Discussion

This study reveals that among the 6 different treatments of mustard oil meal and carriers tested, the ground oil meal of mustard (*B. juncea*) along with carrier C-5A containing the volatile compound is fungicidal to *Alternaria solani*. This study also reveals that the predominant volatile compound allylisothiocyanate is responsible for its anti-fungal properties. Volatile compounds are released as a result of hydrolysis of glucosinolates in the seeds of *Brassica* species (Cole 1980). Among the concentrations tested against potato pathogen *Alternaria solani,* responsible for early blight disease, the concentration at or above 500ppm was found fungicidal to the said pathogen. Previous reports also indicate about the fungicidal properties of allylisothiocyanate, *Phytopthora spp.* were the most susceptible whilst *V. dahliae* was the least susceptible (Robin Harding, SARDI, 2001). Carrier C-5A mixed with the ground mustard oil meal in the ratio of 1:1 releases the volatile compound allylisothiocyanate at 7% treatment among the 6 tested carriers and 11 different combinations. This finding suggests that the type of carrier can affect the release of allylisothiocaynate from the ground mustard oil meal, and carrier C-5A is the most effective carrier among the tested samples for use in the seed treatment for control of *Alternaria solani* early blight of potato. The use of this mixture as seed treatment for the management of *Alternaria solani* in the commercial production of potato crops is economically feasible.

Conclusions

This study reveals that Indian mustard (*Brassica juncea*) oil meal containing bio-active compound allylisothiocyanate can be utilized for controlling the plant pathogenic fungi to a great extent without harming the environment. Further studies in this area are needed to screen most devastating pathogens against allylisothiocyanate.

Acknowledgement

This project was funded by Technology Mission on Oilseed Pulses and Maize (TMOP&M) and Council of Scientific and Industrial Research (CSIR), Govt. of India.

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