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Soil Incorporation of Different *Brassica* spp. Reduces Incidence of Bacterial Wilt Caused by *Ralstonia solanacearum*

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ABSTRACT

Ralstonia solanacearum, a soil borne pathogen is the major threat in cultivation of solanaceous crop which causing bacterial wilt diseases. Bio-fumigation is an alternative control method to suppress soil microorganisms, such as fungal, bacterial pathogens and nematodes. Soil incorporation of Brassica spp. viz., B. olaraceae (cabbage), B. carinata, B. nigra, B. juncea, B. napus, mustard seed cake and B. caulorapa were found effective in reducing the bacterial wilt disease with 86.94 per cent disease control over untreated under glasshouse condition. Field experiments with incorporation of brassica spp. also reduced the incidence of bacterial disease in infested soil. B. olaraceae (cabbage) was found more effective in suppression of bacterial wilt with 69.33 per cent disease over control among the different Brassica spp. treatments undr field condition followed by B. juncea and B. carinata with 66.78 per cent disease control over untreated control at 45 days after planting. Mustard seed cake with 62.81 per cent disease control, Raphanus sativus (radish), B. caulorapa (Knol khol) and B. napus with 57.04 per cent disease control were found next best bio fumigant. In streptomycine (standard check) treated plot.52.28 per cent disease control was observed.

Key words: *Bio-fumigation, Brassica spp., Bacterial wilt.*

INTRODUCTION

Bacterial wilt is the major disease which can cause almost total destruction during the rainy season in all the tomato growing areas. It is caused by *R. solanacearum* (E. F. Smith) Yabucchi *et al.*[22]. This disease is also a major constraint in the production of many other important vegetables, fruits and cash crops *viz*., potato, brinjal, ginger, groundnut, tobacco and banana etc. In tomato, estimated yield loss due to bacterial wilt is range from 15 to 95 per cent[10]. In India losses in yield due to bacterial wilt in tomato is 90 per cent,[17]. Yield losses caused by bacterial wilt (BW) are estimated at 50-100 per cent in the traditional potato, capsicum and banana production areas[7]. It is causing yield loss of over 70% in 60% of the tomato fields of Nigeria[1].

At present, the management of the disease is through genetic resistance and use of antibiotics. However, due to existence of variation in the pathogen, breakdown of resistance is very common.

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Further, the use of antibiotic has a limited control. Hence, an eco-friendly and alternative method for the management of bacterial wilt pathogen, commonly all soil borne pathogens can be done through bio-fumigation by using *Brassica* spp. as bio-fumigant.

Brassica and other members of Brassicaceae contain significant quantities of thioglucoside compounds known as glucosinolates (GSLs) in their tissues[14]. Upon tissue disruption, GSLs are hydrolysed by endogenous myrosinase (thioglucoside glucohydrolase EC3.2.3.1) to release isothiocyanates (ITCs), thiocyanates, nitriles or oxazolidinethiones. The nature of the hydrolysis products depends upon the type of organic side chain (which can be aliphatic, aromatic or indolyl) on the parent molecule and the environmental conditions[18]. GSLs are relatively inactive against microorganisms, but their hydrolysis products, particularly ITCs, are highly biocidal to a diverse range of organisms including nematodes, bacteria, fungi, insects and germinating seeds[5,8,18]. Accordingly, ITCs released from Brassica rotation and green manure crops or seed meal amendments incorporated into soil have the potential to suppress pest and disease organisms[21]. As many ITCs are volatile, biofumigation is a term recently used to describe the suppression of soil-borne pests and pathogens by Brassica crops[3,12,11] and there is considerable interest in biofumigation as an alternative to synthetic soil fumigants in horticulture, and for control of soilborne pathogens in broad acre agriculture[5].

Bio-fumigation is an alternative control method which works on the principle of exploiting the natural biocide compounds from high glucosinolate containing plants[12,13,15] to suppress soil microorganisms, such as fungal, bacterial pathogens and nematodes,[3,5,19,21]. The term was first coined by Kirkegaard *et al.*[11] who specifically described using glucosinolate hydrolysis products, notably isothiocyanates, to control soil borne pests and pathogens in horticulture and agricultural crops. Isothiocyanates are produced during glucosinolate hydrolysis which occurs when *Brassica* plant tissues are broken down, allowing both glucosinolates and a myrosinase to come into contact with each other and hydrolysis to occur. Inturn this releases one of several products, including isothiocyanates.

The glucosinolate content and concentration is known to differ between Brassica cultivars and throughout development[2,4]. GSLs are commonly found to most readily accumulate in all vegetative and reproductive parts throughout plant development[6]. It is well accepted that the efficacy of bio-fumigation is dependent on the specific glucosinolate hydrolysis products formed during tissue breakdown. Different bio-fumigant crops used will potentially have different bio-fumigation potential and produce different levels of pathogen control[16]. In this contest we conducted a field experiment the objective of effect of soil incorporated *Brassica* spp. on incidence if bacterial wilt disease.

MATERIAL AND METHODS

Seeds of different *Brassica* spp. *viz*., *B. juncea, B. carinata*, *B.napus*, *B.nigra*, *B. rapa* and their cultivers were collected from the Directorate of Rapeseed and Mustard, Bharathpur, Rajasthan. Seeds of *B. olaraceae* (cabbage), *Raphanus sativus* (radish), *B. caulorapa* (knolkhol) and *B. olaraceae* var. *italica* (broccoli) were purchased from local market.

1. Glasshouse experiment:

Glasshouse study was taken to evaluate the bio-fumigation potential of *Brassica* species against bacterial wilt (*R. solanacearum*) of tomato. Eleven different *Brassica* species, eight cultivars of mustard and mustard seed cake were used to evaluate their bio-fumigation potential against *R. solanacearum* under glasshouse condition.

1a.Growing of *Brassica* spp. for pot incorporation

Different *Brassica* spp. and cultivars were sown in experimental field. Before sowing the field was ploughed and fine seed bed was prepared, FYM was mixed with soil to increase the fertility. Plants were watered regularly and nutrients were supplied for the good growth. Weeding was done to minimize the competition for nutrients and for good penetration of sunlight.

1b. Pot filling and inoculum development

Soil was collected from the pathogen sick field which had the history of severe bacterial wilt incidence and was filled into plastic pots (8 inch size). Again the soil in each pot was artificially inoculated by adding 10 ml of the *R. solanacearum* inoculum suspension at 1×10^8 CFU/ml to obtain a final estimated population of 2.5×10^8 CFU/g of dry soil.

When the *Brassica* plants attained maximum vegetative growth before flowering they were harvested and brought to the glasshouse. The *Brassica* plants were chopped into small pieces and mixed with the pot soil inoculated with *R. solanacearum* at the rate of 100 tons per ha (120 g per pot). Each treatment was replicated thrice and each replicate contained three plants. The treatments were left for fifteen days for the initiation of bio-fumigation activity of the brassica tissue. Regular watering was done to increase the bio-fumigation efficiency.

After fifteen days, the 25 days old tomato seedlings cv. Shivam was planted in the treated pots. Along with the treatments two controls were kept, one as pathogen control with no treatment and another one as positive control with antibiotic (streptomycin 500 ppm) treatment. Per cent wilt incidence was recorded and compared with the pathogen and positive checks.

1. Field experiment:

Brassica spp. that were effective in reducing the wilt incidence under glasshouse condition were selected for field evaluation.

The experiment was laid out as per Randomized Complete Block Design. *Brassica* plants were raised in the microplots of size 3 × 2 m, when the plant attained maximum vegetative growth before flowering, the tissue was incorporated in to the soil. Irrigation was provided for the release of isothiocyanates and to enhance the biofumigation activity. After 10 days of *Brassica* tissue incorporation, planting was done with 25 days old Tomato seedlings (var. Shivam) with a spacing of 60 × 45 cm. The antibiotic treatment, streptomycine 500 ppm was drenched around the root zone after planting @ 50 ml per plant. Each treatment was replicated thrice and each replication contained 10 seedlings. All the cultural operations, application of fertilizers etc., were followed as per the package of practices.

Treatments imposed in the field trial

T_1: *B. olaraceae* (cabbage)	T_6: *B. junea*
T_2: *Raphanus sativus* (raddish)	T_7: *B. napus*
T_3: *B. caulorapa* (knol khol)	T_8: Mustard seed cake
T_4: *B. carinata*	T_9: Streptomycine@ 500 ppm
T_5: *B. nigra*	T_{10}: Control

Observations on percentage of wilt incidence was recorded on the 7, 14, 21, 30 and 45 days after planting

RESULTS

Glasshouse: The observations on per cent incidence of bacterial wilt in brassica treated pot revealed no symptoms up to seven days of planting under glasshouse condition. However, wilting symptoms were observed in control pots which progressively increased and by 28 days after planting 100 per cent wilt incidence was observed. Among the treatments, *B. olaraceae* (cabbage), *B. carinata*, *B. nigra*, *B. juncea*, *B. napus*, mustard seed cake and *B. caulorapa* were found effective with 86.94 per cent disease control followed by *B. olaraceae* var. *italica* (Broccoli) 65. 63 per cent and Mustard (local), *Raphanus sativus* (radish), *B. juncea*, *B. rapa* (yellow sarson) with 60.82 per cent disease control over untreated control. However, 47.96 per cent disease control was observed in streptomycine treatment (table 1and fig.1).

Table1: Evaluation of *Brassica* spp. against bacterial wilt under glasshouse condition

Treatments		Per cent wilt incidence				Per cent disease control
		7 days	14 days	21days	28 days	
T_1	Mustard (local)	0.00	35.26	35.26	35.26	60.82
T_2	*B. olaraceae* (Cabbage)	0.00	0.00	11.75	11.75	86.94
T_3	*Raphanus sativus* (Radish)	0.00	35.26	35.26	35.26	60.82
T_4	*B. carinata*	0.00	0.00	0.00	11.75	86.94
T_5	*B. nigra*	0.00	0.00	11.75	11.75	86.94
T_6	*B. juncea*	0.00	0.00	0.00	0.00	60.82
T_7	*B. napus*	0.00	0.00	11.75	11.75	86.94
T_8	*B. rapa* (var. RH 119)	0.00	35.26	35.26	35.26	60.82
T_9	*B. rapa* (var.YSH 401)	0.00	35.26	35.26	35.26	60.82
T_{10}	*B. juncea* (var. RH 406)	0.00	41.76	48.25	54.74	39.17
T_{11}	*B. juncea* (var. NRCDR 2)	0.00	35.26	35.26	35.26	60.82
T_{12}	*B. juncea* (var. NRCHB 101)	0.00	35.26	35.26	39.00	56.66
T_{13}	*B. juncea* (var. RH 749)	7.41	35.26	35.26	35.26	60.82
T_{14}	*B. juncea* (var. DRMR IJ31)	0.00	35.26	35.26	35.26	60.82
T_{15}	*B. juncea* (var. Urvashi)	0.00	35.26	35.26	41.76	53.6
T_{16}	Mustard seed cake	0.00	0.00	0.00	11.75	86.94
T_{17}	*B. rapa* (Yellow sarson)	0.00	35.26	35.26	35.26	60.82
T_{18}	*B. caulorapa* (Knol khol)	0.00	0.00	0.00	11.75	86.94
T_{19}	*B. napus* var. *napus* (Gobi mustard)	0.00	35.26	35.26	35.26	60.82
T_{20}	*B. olaraceae* var. *italica* (Broccoli)	0.00	35.26	35.26	30.93	65.63
T_{21}	Streptomycine@500ppm	0.00	35.26	35.26	46.83	47.96
T_{22}	Control	35.26	54.74	66.54	90.00	0.00
SEm±		**1.58**	**1.38**	**5.19**	**5.95**	
CD (0.01)		**6.01**	**5.26**	**19.79**	**22.6**	
CV %		**1.41**	**0.09**	**0.32**	**0.33**	

Wilting of plants in untreated control Plants in bio-fumigants treated pots

Fig. 1: Plants in control pot and bio-fumigants treated pots

Eight *Brassica* spp. which were found effective in suppression of bacterial wilt under glasshouse condition were evaluated in the sick plot. Observations were recorded on the per cent wilt incidence at 7, 14, 21, 30 and 45 DAP and the results are presented in the table2.

Table 1: Field evaluation of different *Brassica* spp. against bacterial wilt disease

Treatment		Per cent wilt incidence					Per cent disease control
		7 days	14 days	21 days	30 days	45 days	
T_1	*B. olaraceae* (Cabbage)	0.00	12.29	21.14	21.14	26.57	69.33
T_2	*Raphanus sativus* (Radish)	0.00	26.07	28.78	33.21	37.22	57.04
T_3	*B. caulorapa* (Knol khol)	0.00	21.14	28.08	30.79	37.22	57.04
T_4	*B. juncea*	0.00	15.00	21.14	21.14	28.78	66.78
T_5	*B. nigra*	0.00	12.29	18.43	21.14	31.00	64.22
T_6	*B. carinita*	0.00	12.29	21.14	21.14	28.78	66.70
T_7	*B. napus*	0.00	21.14	23.86	26.57	37.22	57.04
T_8	Mustard seed cake	0.00	26.07	28.78	35.22	35.22	62.81
T_9	Streptomycine	0.00	28.78	31.00	35.22	43.08	50.28
T_{10}	Control	21.14	43.08	45.00	50.77	86.65	0.00
SEm±		**0.86**	**4.93**	**2.51**	**1.80**	**2.12**	
CD (0.05 %)		**2.57**	**14.79**	**7.54**	**5.40**	**6.36**	
CV %		**20.35**	**19.17**	**16.28**	**10.52**	**9.83**	

Up to 14 days after planting all the bio fumigants treated plot did not showed any wilting symptoms. While, in the control plot wilting of plants were observed at seven days after planting. Among the different *Brassica* spp. treated, *B. olaraceae* (cabbage) was found to be more effective in suppression of bacterial wilt with 69.33 per cent disease over control followed by *B. juncea and B. carinata* with 66.78 per cent disease control over untreated control (Plate 1) at 45 days after planting. The next best bio fumigant was found to be mustard seed cake with 62.81 per cent disease control, *Raphanus sativus* (radish), *B. caulorapa* (Knol khol) and *B. napus* with 57.04 per cent disease control. However, 52.28 per cent disease control was observed in streptomycine treated plot.

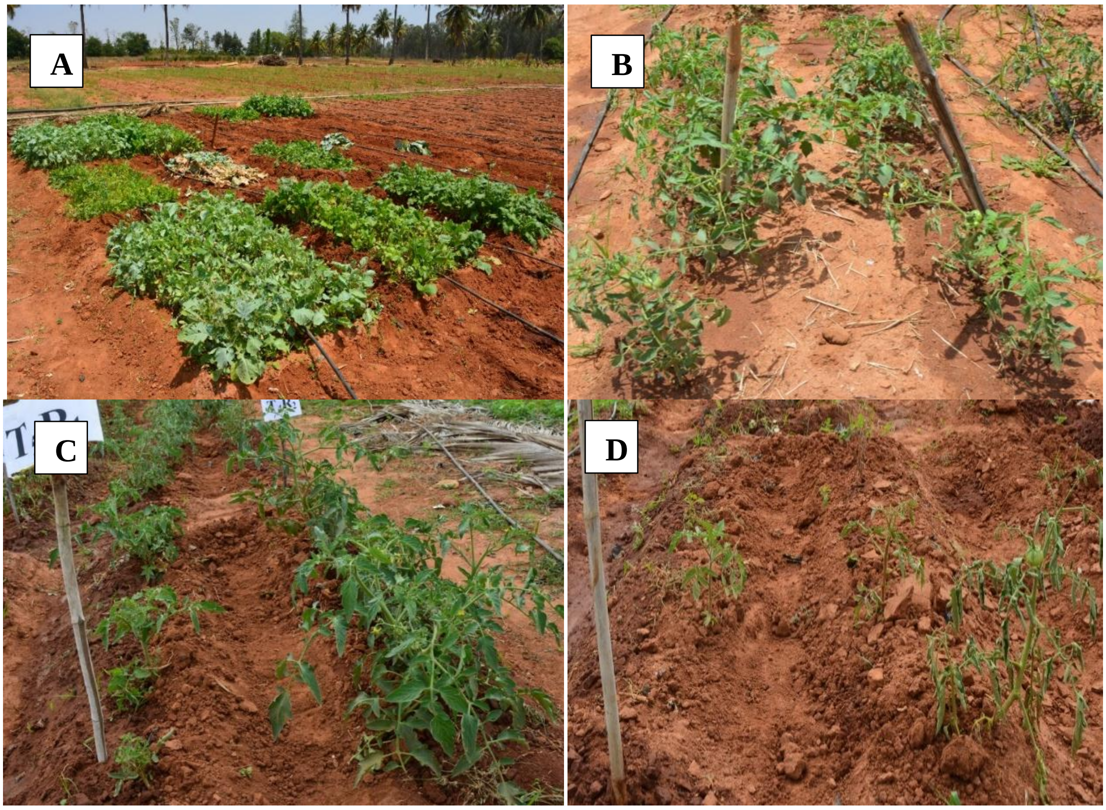

Fig.2: Effect of bio-fumigants on bacterial wilt disease

A: Field view of Brassica spp. before soil incorporation
B: Effect of *B. olaraceae* (Cabbage) on bacterial wilt disease
C: Effect of *Brassica nigra* on bacterial wilt disease
D: Untreated control

DISCUSSION

The glasshouse evaluation of *Brassica* spp. revealed that all the *Brassica* spp. incorporated to soil had suppressive effect on bacterial wilt disease compared to untreated control. Treatments with *B. olaraceae* (cabbage), *B. carinata*, *B. nigra*, *B. juncea*, *B. napus* mustard seed cake and *B. caulorapa* were found effective with 86.94 per cent disease control followed by *B. olaraceae* var. *italica* (broccoli) 65. 63 per cent and Mustard (local), *Raphanus sativus* (radish), *B.juncea*, *B. rapa* (yellow sarson) with 60.82 per cent disease control over untreated control. According to the ACIAR project on biofumigation, using radish, mustard and broccoli reduced bacterial wilt by 50 to 60 per cent. By incorporation of mustard leaves wilt incidence was reduced by 40-80 per cent.

The reduction in per cent wilt incidence is due to the reduction in bacterial population which reduces the inoculum in the soil and will in turn reduce the incidence and severity of disease which was confirmed by soil enumeration of *R. solanacearum*. The reduction in population is due to the toxic effect of GSLs and ITCs released by soil incorporated tissues of *Brassica* spp. We also noticed the minor reduction in the other rhizosphere microflora of the soil but there is no significant difference was observed when compared to control. This may be due to their ability to withstand the effect of GSL's and ITC's released during bio-fumigation process and their ability to utilize organic compounds released by decomposing tissues of *Brassica* spp.

The field evaluation revealed the potential of *Brassica* spp. in the suppression of bacterial wilt. Among the different *Brassica* spp. *B. olaraceae* (cabbage) was found more effective in reducing bacterial wilt with 69.33 per cent control in field condition, this may be due to their thickness and broad leaf surface that contains higher amount of GSLs and ITCs. *B. juncea* and *B. carinata* (66.78 % control) *B. nigra* (64.22 % control), mustard seed cake (62.81 %), *Raphanus sativus* (radish) and *B. caulorapa* (57.04 %) were also found effective and are the next best treatments.

Under field conditions no wilting symptoms were observed up to 14 days after planting in all the treatments, after that gradual wilting of plants were observed. This may be due to the decrease in the biofumigation potential after 25-30 days of incorporation. Probably, the pathogen residing in soil might have helped in build-up of population after 30 days of treatment. Agustin and Floresca also found the reduction in the population of *R. solanacearum* after application of brassica bio fumigants (broccoli and cabbage) in the soil.

In this study, a general observation was made on the growth and phenotypic appearance of tomato plants. In the Brassica treated plots, robust and fast growing compared to control. This may be due to the treated *Brassica* spp. that has accounted for the organic matter content of the soil. Incorporation of bio-fumigants into the soil provides valuable organic matter, possibly reducing the dependence on organic fertilizers. Other benefits of bio-fumigation include improved soil texture, increased water holding capacity and improved microbial community structure[9].

Thus bio-fumigation provide a sustainable disease control option, in integrated BW (Bacterial wilt) management and it is well fit into the organic farming as it improves soil fertility and no toxic residues.

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