APPLICATION OF BIOLOGICAL SOIL DISINFESTATION FOR CONTROLLING VASCULAR WILT DISEASE OF TOMATO

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ABSTRACT

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A field trial was conducted on the potentiality of biological soil disinfestation (BSD) in suppressing wilt diseases of tomato in the research field of Regional Agricultural Research Station, Rahmatpur, Barishal during the 2014-15 and 2015-16 cropping seasons. Four types of biomass, viz., mustard plants, rice bran, Mustard Oil Cake (MOC), and kitchen wastes were incorporated into soil as BSD-treatments, whereas the other two treatments exclude the biomass and /or polythene covers. Among all BSD-treatments; especially for the mustard plants-treatment (TB) reduced the disease incidence to 86-87.19%, and the wilt incidence was 1.97% and 2.18% in respective years. All polythene covered soil including BSD-treated soil showed a significant increase of tomato yield as compared to uncovered soil. Among the treatments, mustard-treated soil gave the highest yield (60.80 and 58.18 t/ha) in both the cropping seasons. Based on results of the present investigation soil covered with TB (covered, irrigated and mustard plants-treated) @ 10 t/ha or (TM) (covered, with MOC and irrigation) @ 500 Kg/ha or (TK) (covered, irrigated and kitchen waste-treated) @ 10 t/ha or (TR) (covered, irrigated and rice bran-treated) @1.25 t/ha; with anaerobic condition with plastic taping maintained for about 2 weeks may be recommended for controlling vascular wilt disease of tomato.

Keywords: BSD, Control, Vascular wilt, Tomato

INTRODUCTION

Tomato *(Solanum lycopersicum* L) is one of the world's most widely cultivated vegetable crops for consumption as fresh fruits and various types of processed products (Hariprasad and Niranjana 2009; Giovannoni *et al*. 2004). Low yield of tomato is attributed to its susceptibility to several pathogenic fungi, bacteria, viruses and nematodes which are major constraints to tomato cultivation worldwide (Barone and Frusciante 2007). Tomato wilt disease caused by fungal pathogens such as *Fusarium oxysporum* f.sp. *lycopersici*, *Verticillium albo-atrum*, and *Sclerotium rolfsii*, bacterial pathogen *Ralstonia solanacearum*, and nematodes (*Meloidogyne incognita* or *M. hapla*) causes severe yield losses in tomato growing areas. It causes damping-off, wilting, root rot, and discoloration of the vascular system of seedlings and mature plants. Although soil fumigation with methyl bromide, chloropicrin, or other chemicals has been used successfully to control the disease, their use has been associated with potentially severe environmental problems or detrimental for human health (Kuniyasu *et al*. 1993; Gina *et al.* 2008).

The chemical control using various pesticides is not eco-friendly and ineffective in most cases. To combat the disease, pre-plant soil disinfestation is essential. Biological soil disinfestation (BSD) may be a suitable option for controlling this disease effectively. Besides incorporating organic matter into the soil BSD not only controls soil pathogens but also suppresses weeds. Biological soil disinfestation (BSD) is a method for controlling soil-borne pests and diseases through anaerobic decomposition of plant biomass that was mainly developed in the Netherlands (Blok *et al.* 2000; Messiha *et al*. 2007) and Japan (Shinmura 2004; Momma 2008). Recently, BSD has become popular in the world, especially in organic agriculture as an alternative to chemical fumigation. For BSD, plant biomass is incorporated into soil followed by the application of irrigation water and covering the soil surface with transparent plastic film for about three weeks to induce reduced soil conditions and to maintain suitable soil temperature

(Shinmura 2000, 2004). Thereafter, crops can be cultivated removing the plastic film and plowing the field. Plant biomass sources such as *Brassicaceae* plants, wheat bran, rice straw, rice bran, *Avena* spp, grasses, or other organic substances have been reported to be used successfully for BSD against soil-borne pests and diseases (Mojtahedi *et al.* 1991; Sarwar and Kirkegaard 1998; Shinmura 2004; Goud *et al.* 2004). *Brassicaceous* cover used for BSD as a promising biomass for incorporation (Stapleton *et al.* 2000) that decompensated to glucosinolates and release of isothiocyanates (ITCs), in addition to thiocyanates, nitrites, and oxazolidinethiones, which are toxic to many soil pathogens (Sawar and Kirkegaard 1998; Fahey *et al*. 2001). Thus, incorporation of the *Brassicaceae* such as *Brassica juncea*, *B. napus*, *B. nigra*, *B. oleracea*, and *B. campestris* used as BSD material were reported to control various soil-borne diseases and crop mortality caused by *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Verticillium* spp. *Alternaria alternata*, *Colletotrichum dematium*, and plant-parasitic nematodes (Tsror *et al.* 2007; Mattner *et al.* 2008; Ramirez *et al.* 2009). Therefore, the research program was undertaken to find out the suitability of BSD in controlling tomato wilt diseases in field conditions in Bangladesh.

MATERIALS AND METHODS

The experiment was carried out at the research field of Regional Agricultural Research Station, Rahmatpur, Barishal during the late rabi season of 2014-15 and 2015-16. The experimental site is located under the agro-ecological zone Ganges Tidal Floodplain (AEZ-13). The soil type is medium high land and soil texture is loamy. The plot size for each treatment was 2.0 × 3.0 m^2. The number of treatments used in this experiment was six distributed in a randomized complete block design with three replications. The recommended doses of manures and fertilizers for tomato were incorporated into soil. The soil was treated with various plant biomass sources such as mustard plants @ 10 t/ha (TB), rice bran @1.25 t/ha (TR), MOC 500 Kg/ha, and kitchen wastes @ 10 t/ha (TK) together with applying irrigation water and covering with black polythene to maintain strictly anaerobic condition. The name of treatments TB (covered, irrigated and mustard-treated); TK (covered, irrigated and kitchen waste-treated); TR (covered, irrigated and rice bran-treated); TM (covered, with MOC and irrigation); TW (covered, no biomass and irrigated); and TC (uncovered and no plant biomass). For the treatments TB, TR, TK, TM, and TW, anaerobic condition with plastic taping was maintained for about 2 weeks. After that, holes were made at a spacing of 40 × 40 cm^2 on the black polythene to degas the treated soil. Tomato seedlings (BARI Tomato -3) grown in the nursery bed were transplanted into the holes of the polythene sheet and the total number of plants per plot was 28. All the plots were inspected regularly and intercultural operations such as watering, weeding, and insect control were done as and when necessary. During the establishment of the seedlings, a lot of winged aphids were available in the tomato field; hence yellow traps (plastic bottle stained with yellow color and Mobil oil) were set in the field to trap the insects. The natural wilt disease incidence was recorded in all plots at 15 days interval. Further, Plant height (30 DAP and 65 DAP), number of fruits/plant, and yield/plot were recorded. Statistical analyses were performed using MSTAT-C and Microsoft Office Excel 2010.Treatments were compared via ANOVA using the least significant differences test (LSD) at 5% ($P \leq 0.05$) probability level.

RESULTS AND DISCUSSION

The results of the study are presented in Table 1. The natural incidence of wilt diseases was observed in the tomato plots. A smaller number of tomato plants were infected by the wilt disease in most of the plots. All BSD treatments (TB, TK, TR, and TM) significantly (P=0.05) reduced the disease incidence to 30.88-87.19%, especially for the mustard-treatment (TB) the wilt incidence was only 1.97% and 2.18% and untreated control plots (TC) exhibited 15.38 and 15.57% during the cropping seasons 2014-15 and 2015-16 respectively. When considering marketable yield, all polythene covered soil including biomass-treated soil resulted significant increase of tomato yield as compared with uncovered soil (TC). Among the treatments, mustard-treated soil (TB) showed the highest yield (60.80 and 58.18 t/ha) followed by TM (54.58 and 55.53 t/ha) and TK (53.11 and 53.88 t/ha). However, the treatment TC (uncovered and no plant biomass) gave the lowest tomato yield (38.86 and 40.82 t/ha). Every year, the maximum increase in yield was achieved with mustard-treated soil (TB)followed by MOC treated soil (TM), kitchen waste treated soil (TK). The increase in this parameter was higher during 2014-2015 than 2015-2016 (Table 1).

The data on the most important yield contributing parameters such as plant height and number of fruits per plant were recorded during 2014-2015 and 2015-2016 crop seasons. Average plant height and number of fruits per plant ranged 19.80-29.83 cm at 30 DAP; 54.67-63.23 cm at 65 DAP and 10.38-17.60

fruits/plant, under different treatments including control.

Table 1. Effect of BSD on disease incidence, yield and yield contributing characters of tomato

Treatments	Plant height (30 DAP) (cm)		Plant height (65 DAP) cm		Number of fruits/plant		Yield (t/ha)		% Wilt infected plants	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
TB	28.67 a	29.83 a	62.40 a	63.23 a	16.01 a	15.60a	60.80 a	58.18 a	1.97 e	2.18 e
TK	27.67 a	28.57 ab	59.07 ab	59.37 ab	15.13 a	14.40ab	53.11bc	53.88 ab	10.10 c	9.22 c
TR	26.40ab	27.47 ab	59.07 ab	59.90 ab	14.82 a	14.03ab	52.05bc	51.19abc	10.63 c	10.42 c
TM	28.27 a	29.17 a	59.93 ab	60.17 ab	15.63 a	15.27a	54.58 b	55.53 ab	7.07 d	6.10 d
TW	24.40 b	25.30 b	57.93 ab	58.33 ab	14.00 a	12.73b	48.64 c	48.50 b	13.30 b	13.10 b
TC	19.80 c	21.10 c	54.67 b	56.33 b	10.38 b	12.5b	38.86 d	40.82 c	15.38 a	15.57 a

Means within the same column with a common letter(s) are not significantly different at 5% level of probability TB=covered, irrigated, and mustard-treated. TK= covered, irrigated and kitchen waste-treated; TR= covered, irrigated and rice bran-treated; TM= covered, with MOC and irrigation; TW= covered, no biomass and irrigated; TC= uncovered and no plant biomass.

Table 2: Soil status and fungal colony count in differently treated soils

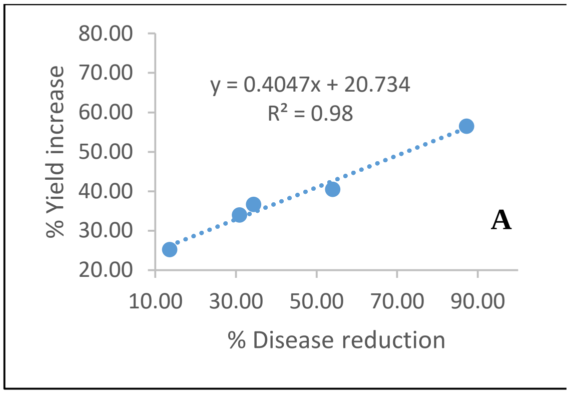
Treatments	Moisture content (%)		Total fungal colony count (CFU/g dry soil)	
	2014-15	2015-16	2014-15	2015-16
TB	30.2	30.3	4.40×10^5	4.50×10^5
TK	31.2	31.4	9.40×10^5	9.30×10^5
TR	29.3	29.5	9.12×10^5	9.05×10^5
TM	29.0	29.2	4.60×10^5	4.58×10^5
TW	30.0	29.8	3.33×10^6	3.23×10^6
TC	27.0	26.9	5.50×10^6	5.62×10^6

TB=covered, irrigated, and mustard-treated. TK= covered, irrigated and kitchen waste-treated;TR= covered, irrigated and rice bran-treated; TM= covered, with MOC and irrigation; TW= covered, no biomass and irrigated; TC= uncovered and no plant biomass.

All bio-mass treated plots and single polythene covered plots caused a significant increase in plant height and number of fruits per plant over control. Both growth parameters are the most important yield attributes of tomato. The highest increase in plant height of 28.67 and 29.83 cm at 30 DAP and 62.40 and 63.23 cm at 65 DAP were found under TB followed by TM and TK. The lowest increase of the parameter was obtained with TR followed by TW (Table 1).

In both seasons of the experiment, the relationship of reduction in severity of vascular wilt and marketable yield of tomato due to treatment effect was linear, positive, and significant (r=0.989* and r=0.976*). Their relationship could be expressed by the regression equations, Y=0.4047x+20.734 in 2014-15 and Y=0.3331x+15.198 in 2015-16. The coefficient of determination (R^2) indicates that yield increase due to reduction in disease severity may be attributed to 87.19% in 2014-15 and 86.00% in 2015-16 (Fig. 1).

On the other hand, results revealed that (Table 2), for the non-treated soil, the total fungal population were 4.40 × 10^6 and 4.50× 10^6CFU/g soil and the highest amount 5.50× 10^6 and 5.62× 10^6CFU/g soil were detected from the untreated control. All BSD-treated soils showed a reduced number of fungal colonies as compared to the control. The results indicated that the total fungal population declined after the BSD treatment.



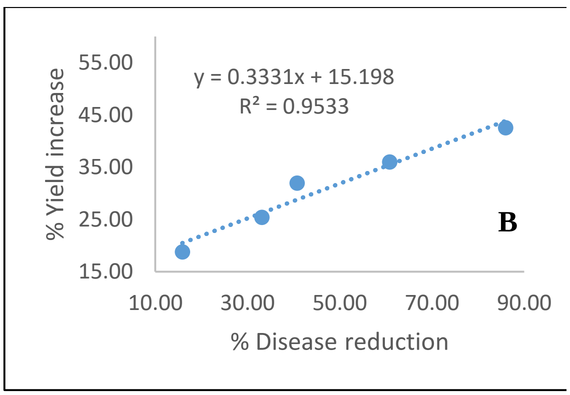


Fig. 1. Relationship of percent yield increase of tomato with percent wilt disease reduction during 2014-2015 (A) and 2015-2016 (B) crop seasons

Results of the present experiment reveal that the treatments TB, TR, TK, TM, and TW effectively reduced vascular wilt severity and substantially increased the yield of tomato. The increased yield performances of tomato in the BSD-treated fields (TB, TM, TR, and TK) might be attributed to the suppression of wilt disease, conservation of soil moisture, effective control of weeds, and addition of fresh biomass or organic matter in soil. For the polythene covered soils TW, the suppression of weeds and moisture conservation might play an important role in increasing the yield. The result showed similarity with the previous finding (Mowlick *et al.* 2012, 2013a), where the number incorporated *Fusarium oxysporum* pathogens (wilt pathogen of tomato and spinach) decreased significantly after BSD treatment in pots. *Brassicaceae* plants are known to contain bioactive substances and have been widely used for bio-fumigation in soil (Kirkegaard *et al*. 1996; Larkin and Griffin 2007). BSD treatments with mustard greens (*B. juncea* var. *cernua*) and *Avena* plants, as well as wheat bran, successfully controlled the population of the pathogens (*F. oxysporum* f. sp. *lycopersici*, wilt pathogen of tomato, and *F. oxysporum* f. sp. *Spinacheae* wilt pathogen of spinach were evaluated by Mowlick *et al*. (2012, 2013) in two pot experiments using soil from different districts of Japan. For the *Brassica*-treated soil, ITCs from the tissue damage of *Brassica* leaves have been mentioned as the major causal factors to kill the pathogens (Mattner *et al*. 2008).

Apart from the function of glucosinolates, studies have demonstrated that the pesticidal activity of *Brassica* spp is likely due to other factors such as releasing of aldehydes, acids, and other sulfur- and nitrogen-containing compounds during plant growth or biomass decomposition in soil (Gamliel and Stapleton 1993). Soil incorporated with *B. juncea* plants, wheat bran, or radish biomass suppresses the *Fusarium oxysporum* pathogen as well as natural wilt disease incidence (Mowlick *et al*. 2012, 2013, 2013a, 2014). All these treated soils contained volatile bioactive substances and bacteria in the *Firmicutes* phylum dominate the soil. Thus, it was suggested that anaerobic bacterial decomposition of plant biomass release a bioactive substance that suppresses the soil-borne pathogens.

Based on results of the present investigation it may be concluded that soil covered with TB (covered, irrigated and mustard-treated) @ 10 t/ha; TK (covered, irrigated and kitchen waste-treated) @ 10 t/ha; TR (covered, irrigated, and rice bran-treated) @1.25 t/ha; TM (covered, with MOC and irrigation) @ 500 Kg/ha, with anaerobic condition with plastic taping maintained for about 2 weeks are effective to control vascular wilt disease of tomato.

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