Vegetable Science (2017) 44 (1) : 103-106

Morphological characterization, cross infectivity and chemo-sensitivity of *Sclerotinia sclerotiorum* isolates towards bio-agent and new molecules of fungicides

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Received: June 2017 / Accepted: July 2017

Abstract

Six different isolates of *Sclerotinia sclerotiorum* were isolated from vegetable host crops namely brinjal, cauliflower, French bean, and pea on potato dextrose agar. These isolates were subjected for morphological characterization, cross infectivity/pathogenicity test and chemo-sensitivity towards bioagent and new molecules of fungicides. On the basis of morphological characteristics *viz.*, colony colour and texture, the tested isolates were categorized into two groups. Cross infectivity / pathogenicity test were performed on French bean, dolichos bean and pea using leaf detachment technique. Results on cross infectivity revealed non-host specificity of tested isolates of the pathogen. As regards to chemo- sensitivity of these isolates with tebuconazole 250 EC, azoxystrobin 23 SC, fluopyram 17.7+ tebuconazole 17.7 SC, Fluopicolide 6.25 %+Propamocarb Hydrochloride 62.5% SC at concentration of 250, 500, 1000 and 1500 ppm, the combination fungicide fluopyram 17.7+ tebuconazole 17.7 SC was found most effective at concentration of 250 ppm against tested isolates of *S. sclerotiorum.* In case of *in vitro* evaluation of bioagent, *Trichoderma asperellum* against these isolates, the highest mycelial growth inhibition (50.46%) against pea isolate (PSS-1) and the lowest inhibition (26.15%) in case of French bean isolate (BSS-1) was observed. Cross infectivity and chemo sensitivity of the pathogen clearly indicate the emergence of this pathogen as broad spectrum covering wide host range.

Keywords: Cross infectivity, Confrontation test, Fungicide, *Sclerotinia* rot, *Trichoderma*

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Introduction

Sclerotinia sclerotiorum is a necrotrophic ascomycete fungus causing stem, fruit and pod rot on a wide range of economically important plant species belonging to different genera of botanical families in a global perspective (Mundkur 1934; Boland and Hall 1994; Lithourgidis et al. 2003; Tripathi et al. 2014). Profiling, documentation and quantification of vegetable diseases is essential for formulation of effective disease management strategies. During December and January of 2015-16, its incidence has been recorded on brinjal, bottle gourd, cauliflower, French bean, dolichos bean and pea in farmers' field and research farm of ICAR-IIVR, Varanasi, Uttar Pradesh. *Sclerotinia* rot has become a major emerging threat to commercial cultivation of major cucurbitaceous, cole, leguminous and solanaceous vegetables (Tripathi et al. 2016). Initial disease symptoms appeared in the form of water-soaked lesions on stem during the flowering and pod formation stages under cool humid conditions. Later, it spreads rapidly on infected plant parts including leaf, flower, fruits and pods finally causing girdling and rotting of these plant parts. Lesions of infected plant parts usually developed patches of white, fluffy mycelial mats, often with large, irregular, black-coloured sclerotia. Management of *Sclerotinia* rots difficult due to its stubborn survival in the soil as sclerotia for many years and the ineffectiveness of available control measures. Considering the difficulties of management of sclerotinia rot in vegetables, the present work was planned with the objective to characterize cross infectivity/host specificity of *S. sclerotiorum* isolates and its sensitivity towards bioagent and novel molecules of fungicides.

Materials and Methods

Pathogen isolates used in study: Infected plant samples of vegetable hosts viz. brinjal, cauliflower,

French bean and pea were collected from the Research Farm, ICAR-IIVR, Varanasi. Diseased stem tissue was surface sterilised for one minute in 1% mercuric chloride. The stem pieces were plated on potato dextrose agar (PDA) and incubated at 20±2°C for 10 days. Pure cultures of pathogen from brinjal (BSS-1), cauliflower (CSS-1), French bean (FSS-1, FSS-2 and FSS-3) and pea (PSS-1) were established on PDA by hyphal tip method. Six isolates of the pathogen were maintained on PDA slants at 20±2°C for further use (Table 1).

Table1: Details of *S. sclerotiorum* isolates used in study

Common name	Botanical name	Isolate code
Brinjal	*Solanum melongena*	BSS-1
Cauliflower	*Brassica oleracea* var *cauliflora*	CSS-1
French bean	*Phaseolus vulgaris*	FSS-1, FSS-2, FSS-3
Pea	*Pisum sativum*	PSS-1

Cross infectivity/host specificity test of *S. sclerotiorum* on isolates on legume hosts: Different isolates of *S. sclerotiorum* (BSS-1, CSS-1, FSS-1, FSS-2, FSS-3 and PSS-1) were subjected to cross infectivity test on different leguminous hosts viz. pea, cowpea, dolichos bean, French bean, sword bean and kevach adopting leaf detachment method. Under *in vitro* conditions, the surface sterilized leaf and pod of these leguminous vegetables were aseptically inoculated with 5 mm mycelial bit of five days old pathogen culture and incubated for 48 h at 18±1°C.

***In vitro* chemo sensitivity of *S. sclerotiorum* isolates towards fungicides and ICAR-IIVR isolate of *Trichoderma asperellum*:** Chemo-sensitivity of isolates were tested with four systemic fungicides namely tebuconazole 250 EC, azoxystrobin 23 SC, fluopyram 17.7+ tebuconazole 17.7 SC, fluopicolide + propamocarb hydrochloride 68.75 SC at concentrations of 250, 500, 1000 and 1500 ppm by poisoned food technique (Nene and Thapaliyal, 2000). *In vitro* confrontation test was done on isolates of *S. sclerotiorum,* stem rot pathogen with isolate of *T. asperellum*.

Results and Discussion

Morphological characterization of pathogen isolates: After incubation, all pathogen isolates produced colonies with abundant, spherical to irregular, large, black-coloured sclerotia on PDA. Germinated sclerotia produced white-coloured colonies with hyaline, septate, branched hyphae. The isolated fungus was identified as *S. Sclerotiorum* based on morphological and cultural characteristics of the mycelia and sclerotia (Purdy, 1979; Willetts and Wong, 1980; Bolton *et al*. 2006). On the basis of morphological characteristics viz. colony colour and texture, tested isolates were categorized into two groups. Cauliflower isolate (CSS-1) producing light white coloured colony with loose texture were kept singly in one group while isolates (BSS-1, FSS-1, FSS-2, FSS-3 and PSS-1) producing bright white coloured colony with compact texture were kept in another group. Cauliflower isolate (CSS-1) produced lowest number of black coloured spherical sclerotia (4) while French bean isolate (BSS-1) produced highest number of sclerotia (55) per plate on PDA (Table 2). The cultural characteristics of pathogen isolates were in conformity with the observation recorded for *S. sclerotiorum* by Hansda et al. (2014).

Cross infectivity/pathogenicity test of isolates of *S. sclerotiorum* on legume host: Cross infectivity test revealed that inoculated *S. sclerotiorum* isolates infected leaves of all the tested leguminous hosts viz. cowpea, dolichos bean, French bean, sword bean, pea and kevach, with maximum severity on cowpea leaf (Fig 1). However, infection level was more on pods of dolichos bean than that of pea (Fig. 2). It clearly revealed cross infectivity and non host specificity of the pathogen indicating the emergence of the fungus as broad spectrum pathogen with wide host range. Our results were in tandem with the results of earlier workers who had reported wide host range existence and non host specificity in *S. sclerotiorum* (Bolland1997; Bag 2000; Lithourgidis et al. 2003; Kumar et al. 2003; Mondal et al. 2012).

In vitro chemo sensitivity of *S. sclerotiorum* isolates towards fungicides and ICAR-IIVR isolate of *T. asperellum*:

Fig. 1: Differential level of infection on leaves by *S. sclerotiorum*

Fig. 2: Detached pod infection assay of *S. sclerotiorum*on

All tested fungicides were able to reduce the mycelial growth and sclerotia production of test isolates of *S. sclerotiorum*. Variable degree of chemo-sensitivity of *S. sclerotiorum* isolates were revealed towards tested systemic fungicides namely tebuconazole 250 EC, azoxystrobin 23 SC, fluopicolide + propamocarb hydrochloride 68.75 SC and fluopyram 17.7+ tebuconazole 17.7 SC at the tested concentrations. Among tested fungicides, fluopyram 17.7+ tebuconazole 17.7 SC was found most effective even at lower concentration (250 ppm) for complete inhibition of mycelial growth and sclerotia production in tested isolates of the pathogen. All test isolates were found highly sensitive for varied mycelial growth (inhibition 75-87.5%) and complete inhibition of sclerotia production with tebuconazole 250 EC (250 ppm) was observed. French bean *S. sclerotiorum* isolate FSS-1 was found least sensitive and isolate FSS-2 and FSS-3 were found insensitive towards fluopicolide + propamocarb hydrochloride 68.75 SC at 250 ppm. Azoxystrobin 23 SC was found effective for complete inhibition of sclerotia production in S. *sclerotiorum* isolate FSS-1, PSS-1 and BSS-1 but was unable to do so against FSS-2, FSS-3 and CSS-1 (Table 2). Fungicides sensitivity and their efficacy in chemical control of *S. sclerotiorum* is well documented (Poter and Philipps 1985; Mueller *et al*. 2002; Russell 2004).

Fungicide sensitivity is known in *S. sclerotiorum* but these new molecules under this study have been not documented anywhere against *S. sclerotiorum*. Efficacy of tebuconazole and azoxystrobin against stem rot caused by *Sclerotium rolfsii* in peanut under field condition was evaluated. *S. sclerotiorum* is homothallic non-conidia forming fungus with lower evolutionary potential and so these plant pathogens associate with lower risk of developing fungicide resistance. Farmers mostly depend on fungicide for management of *Sclerotinia* rot in vegetables. Thus, the monitoring of the sensitivity of *S. sclerotiorum* towards fungicides is essential for its effective management.

In vitro confrontation test between four *S. sclerotiorum* isolates (FSS-3, PSS-1, BSS-1 and CSS-1) and *T. asperellum* isolate exhibited mycelial growth inhibition (Fig. 3). The highest mycelia growth inhibition (50.46%) revealed by *T. asperellum* was against pea *S. sclerotiorum* isolate (PSS-1) and lowest (26.15%) for brinjal *S. sclerotiorum* isolate (BSS-1) after 7th day of incubation at 18±1 ^{o}C. Bolland (1997) observed antagonistic effect

Table 2: *In vitro* chemo sensitivity of *S. sclerotiorum* isolates towards new fungicides and ICAR-IIVR isolate of *T. asperellum*

Treatment	Con. (ppm)	Isolates											
		FSS-1		FSS-2		FSS-3		PSS-1		BSS-1		CSS-1	
		*MI%	*Sc	MI%	Sc	MI%	Sc	MI%	Sc	MI%	Sc	MI%	Sc
Fluopicolide + Propamocarb hydrochloride 68.75 SC Proper formulation as given in MM	250	15.62	40	0	52	0	50	21.87	50	34.35	55	9.37	46
	500	18.75	38	6.25	39	0	50	21.87	46	50	42	21.87	36
	1000	25	32	28.12	13	9.37	37	25	43	59.37	23	25	36
	1500	34.37	33	62.5	8	28.12	32	50	39	75	0	28.12	30
Tebuconazole 250 EC	250	75	0	75	0	81.25	0	81.5	0	81.25	0	75	0
	500	87.5	0	81.25	0	84.37	0	81.25	0	81.25	0	81.25	0
	1000	87.5	0	87.5	0	87.5	0	81.25	0	81.25	0	87.5	0
	1500	87.5	0	87.5	0	87.5	0	87.25	0	87.5	0	87.5	0
Azoxystrobin 23 SC	250	50	0	0	0	12.5	35	75	0	50	0	25	6
	500	56.25	0	50	0	25	20	81.25	0	75	0	56.25	4
	1000	75	0	75	0	50	17	87.5	0	75	0	58.75	0
	1500	81.25	0	81.25	30	50	0	87.5	0	87.5	0	62.5	0
Fluopyram 17.7+Tebuconazole 17.7 SC	250	0	0	0	0	0	0	0	0	0	0	0	0
T.asperellum		NT	-	NT	-	36	4	50.46	2	26.15	3	50.36	3
Control		0	77	0	77	0	77	0	77	0	77	0	77

*MI= mycelial growth inhibition; *Sc = Sclerotia; NT= Not Tested

Fig. 3: Confrontation test between *T. asperellum* and *S. sclerotiorum*

of *T. viride* on white mold in bean plants. In the past many workers reported antagonism and antagonistic mechanism of *Trichoderma* spp. against soil borne fungal plant pathogen (Elad 2000; Mukharjee et al. 2015).

सारांश

सब्जी आतिथ्य फसलों जैसे– बैंगन, फूल गोभी, फराश बीन तथा मटर से *स्क्लेरोशियाना स्क्लेरोशियम* के छः विभिन्न विलगों को पोटैटो डेक्सट्रोज अगर पर अलग किये गये। इन विलगों को जैवकर्ता व नवीन कवकनाशकों के प्रति अकारकीय लक्षण रूपी विवरण संकरण संदूषण/रोगजनक करण परीक्षण व रसायन संवेदनशीलता को आंका गया। अकारकीय लक्षण वर्णन जैसे– उपनिवेश, रंग व संरचना के आधार पर विलगों को दो समूहों में विभक्त किया गया। संकरण संदूषण/रोगजनक करण परीक्षण फराश बीन, सेम व मटर पर लीफ डिटैचमेन्ट तकनीकी से किया गया। संकरण संदूषण से स्पष्ट हुआ कि रोगजनक विलगों का परीक्षण अ–आतिथ्य विशिष्ट था। इन विलगों का रसायन संवेदनशीलता टेबुकोनाजोल 250 ई.सी. , एजोक्सी स्ट्रोबिन 23 एस.सी., फ्लुओपयराम 17.7 + टेबुकोनाजोल 17.7 एस.सी, फ्लुओपिकोलाइड 6.25 प्रतिशत + प्रोपामोकार्ब हाइड्रोक्लोराइड 62.5 प्रतिशत एस.सी. की सान्द्रता 250, 500, 1000 व 1500 पी.पी.एम. पर संयुक्त कवकनाशी फ्लुओपयराम 17.7 + टेबुकोनाजोल 17.7 एस.सी. 250 पी.पी.एम. की सांद्रता पर एस. स्क्लेरोशियम विलगों के परीक्षण से सबसे प्रभावी पाया गया। कषत्रिम परिवेशीय जैवकर्ता मूल्यांकन में *ट्राइकोडर्मा एस्पेरेल्लम* का इन विलगों के प्रति कवक तंत्र विकास में रूकावट (50.46 प्रतिशत), मटर विलग (पी.एस.एस.–1) के प्रति तथा सबसे कम रूकावट फराश बीन विलग (बी.एस.एस.–1) के प्रति (26.15 प्रतिशत) पाया गया। रोगजनकों का संकरण संदूषण व रसायन संवेदनशीलता से स्पष्ट हुआ कि इन रोगजनकों का उच्च आर्विभाव वर्णक्रम अनेकों आतिथ्य को आवरण करते हैं।

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