Fungicide tolerance management of isolates of rice sheath blight pathogen (*Rhizoctonia solani* Kühn)

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An experiment was conducted with ten *Rhizoctonia solani* (RS) isolates, obtained from rice grown at ten diverse geographical locations, to test their level of tolerance to five commonly used fungicides [Tilt 25 EC (Propiconazole, 25 %), Contaf 5 EC (Hexaconazole, 5%), Indofil M – 45 WP (Mancozeb, 75%), Bavistin 50 WP (Carbendazim, 50%) and Monceren 250 SC (Pencycuron, 22.9 %)] and to suggest suitable fungicide(s) for the management of high fungicide tolerant isolate(s). Results showed that isolates had 1.4 - 13.4 times tolerance to Propiconazole (highest in RS – 1 and lowest in RS – 2), 3.4 - 64.2 times to Pencycuron (highest in RS – 5 and lowest in RS – 1), 1.1 - 2.0 times to Carbendazim (highest in RS – 8 and lowest in RS – 11), 1.2 - 2.9 times to Mancozeb (highest in RS – 2 and lowest in RS – 9) and 1.2 - 6.9 times to Hexaconazole (highest in RS – 4 and lowest in RS – 6) over the most sensitive isolate. It was evident from the results that RS – 1, RS – 2, RS – 4, RS – 5, RS – 6, RS – 8, RS – 9 and RS – 11 though had high/ moderate tolerances to Propiconazole, Mancozeb, Hexaconazole, Pencycuron, Pencycuron, Carbendazim, Pencycuron and Propiconazole respectively but were sensitive to Pencycuron, Propiconazole, Carbendazim, Carbendazim / Mancozeb, Hexaconazole, Pencycuron / Hexaconazole, Mancozeb and Hexaconazole/ Pencycuron/ Carbendazim/ Mancozeb respectively. For the management of these high/ moderate tolerant isolates, the above mentioned fungicides against which they were less tolerant/ sensitive could be suggested for alternate or combined but not repeated application to avoid resistance development.

Key words: Fungicide, management, *Rhizoctonia solani*, tolerance

INTRODUCTION

Rice sheath is attacked by nine fungal species belong to six fungal genera. Of them, *Rhizoctonia* with four fungal species is the most dominant fungal genus causing four diseases of rice sheath. *Rhizoctonia solani* is the most important, devastating and yield limiting *Rhizoctonia* species attacking sheath, hampering rice production since last two decades (Kobayashi *et al.*, 1997) and causing crop loss to the extent of 5.9 – 69.0% (Venkat Rao *et al.*, 1990; Naidu, 1992). The incidence and severity of this disease differ among countries, regions, geographical areas and even locations. The reasons for such variation in disease severity have been attributed to the virulence of the pathogen, variations in host genotype, prevalence of congenial soil and plant environment, improper choice of

fungicide(s), dose, time and method of application and faulty cultural practices. Among these, pathogenic variability of *R. solani* is considered as one of the most important cause of varying degree of disease intensity and severity (Taheri *et al.*, 2004; Panja *et. al*, 2011) and such variations have been found even within a field (Singh *et al.*, 2003). It may give advantage to the fungus to attack a vast array of host genotypes, to gain greater survival capacity and to acquire differential fungicide tolerance. Acquisition of these properties not only poses serious threat to manage the disease by cultural, biological and chemical means but also through the development of resistant varieties. Sometimes, a particular fungicide(s) used against sheath blight disease does not give desired level of control in certain location(s). One of the reasons for such failure in control may be the presence of fungicide

resistant / tolerant *R. solani* isolates. So, to make any fungicide resistance management programme fruitful, the study on population structure of *R. solani* in a particular area and their tolerance level to a particular group(s) of fungicides are the vital consideration. Keeping the above background in mind an experiment (*in vitro*) has been conducted to identify appropriate fungicide(s) for sole or mixed or alternate application schedule for fungicide resistance management of *R. solani* isolated from different geographical locations.

MATERIALS AND METHODS

For the present experiment, pathogenicity established and virulence proved ten *R. solani* (RS) isolates, obtained from rice grown at ten geographical locations under five districts in West Bengal (Panja *et al.*, 2011), were selected from laboratory stock culture. Level of tolerance of these isolates to five different fungicides [Tilt 25 EC (Propiconazole, 25 %), Contaf 5 EC (Hexaconazole, 5%), Indofil M – 45 WP (Mancozeb, 75%), Bavistin 50 WP (Carbendazim, 50%) and Monceren 250 SC (Pencycuron, 22.9 %)] having four different concentrations (ppm) [Propiconazole – 0.5, 1.0, 5.0 and 10.0; Hexaconazole – 0.1, 0.25, 0.5 and 1.0; Mancozeb -5.0, 10.0, 25.0 and 50.0; Carbendazim – 0.25, 0.5, 1.0 and 5.0; Pencycuron – 0.1, 0.5, 1.0 and 5.0] fungicides were tested *in-vitro* following poisoned food technique proposed by Shervelle (1979). Different concentrations of fungicides pipetted out from the stock solution were mixed with sterilized, melted PDA medium before plating to obtain the desired concentrations of active ingredient. Twenty millilitre of the fungicide poisoned medium was poured into each sterilized Petri plate. Suitable check was maintained by pouring same volume PDA medium without fungicides into Petri plate. Then nine millimeter (diam) mycelial discs were cut out from the periphery of 72 hrs. old actively growing culture and placed at the centre of the Petri plates and incubated at 28 ± 1 °C temperature in BOD till the full growth of the fungus in control was reached. Three replications were maintained for each fungicide concentration as well as the control. Radial growth of fungal isolates in different concentrations including control was recorded. Extent of inhibition of mycelial growth by each fungicide was calculated by estimating the per cent reduction in mean mycelial radial growth over that of control (Vincent, 1947).

Thereafter, effective concentration in log scale for 50% growth inhibition (EC-50) of each isolate by the fungicide was determined initially from a regression equation, $Y = a + bx$, derived from the log values of fungicide concentration (ppm) as dependent variable (Y) and the probit values of per cent growth inhibition (%) as independent variables (x) [b = regression co- efficient/ slope, a = intercepts]. If the probit value of 50% growth inhibition was put in place of 'x', the corresponding log value of effective concentration of fungicides would be obtained. Then taking the antilog of log of concentration of fungicide, the effective concentration for 50% growth inhibition (EC-50) in ppm scale was finally worked out. The fitness of all the simple regression equations was judged comparing the level of significance with the simple correlation coefficient (r) table value at 5% (r = 0.95) or 1 % (r = 0.99) level for 2 degree of freedom.

RESULTS AND DISCUSSION

Results of the experiment indicated that *R. solani* isolates collected from different geographical regions varied in tolerance to fungicides measured by effective concentration for 50% growth inhibition (EC50). A particular isolate also showed discriminatory tolerance response to five fungicides tested (Table 1). Ten *R. solani* isolates showed wide range of tolerance to different fungicides. The lowest and the highest tolerances as judged by high and low EC 50 values of the isolates were found in RS - 1 and RS - 2 under Propiconazole, in RS - 4 and RS - 6 under Hexaconazole, in RS - 2 and RS - 9 under Mancozeb, in RS - 8 and RS - 11 under Carbendazim, in RS - 5 and RS - 1 under Pencycuron. RS - 1 exhibited 13.4 times higher tolerances to Propiconazole than low tolerant isolate RS - 2 (Fig. 1). Similarly, isolate RS - 4 had 6.9 times higher tolerance to Hexaconazole than low tolerant RS - 6 (Fig. 2), RS – 2 with 2.9 times higher tolerance to Mancozeb than low tolerant RS - 9 (Fig 3), RS - 8 with 2.0 times higher tolerance to Carbendazim than low tolerant RS - 11 (Fig 4) and RS -5 with 64.2 times higher tolerance to Pencycuron than low tolerant RS - 1(Fig 5). It was evident from the results that RS – 1, RS – 4, RS – 2, RS – 8 and RS – 5 though had high tolerances to Propiconazole, Hexaconazole, Mancozeb, Carbendazim and Pencycuron but were sensitive to Pencycuron, Carbendazim, Propiconazole, Pencycuron / Hexaconazole and Carbendazim / Mancozeb respectively (Table 1). Besides, RS – 6 having high tolerance to Pencycuron was sensi-

Table 1: EC-50 (µg/ ml) and tolerance ranking of different *R. solani* (RS) isolates under different fungicide treatments

Isolates	Propiconazole	Hexaconazole	Mancozeb	Carbendazim	Pencycuron	Rank total
RS-1	4.385 (1)*	0.273 (3)	18.197 (4)	0.871 (5)	0.018 (10)	23
RS-2	0.327 (10)	0.310 (2)	23.067 (1)	0.873 (4)	0.251 (5)	22
RS-4	3.436 (3)	0.400 (1)	13.152 (8)	0.570 (9)	0.201 (6)	27
RS-5	1.406 (5)	0.260(4)	17.258 (6)	0.776 (6)	1.156 (1)	22
RS-6	0.465 (9)	0.058 (10)	17.579 (5)	0.655 (8)	1.119 (2)	34
RS-7	0.975 (7)	0.238 (5)	19.364 (3)	0.902 (3)	0.061 (9)	27
RS-8	0.962 (8)	0.077 (8)	22.700 (2)	1.057 (1)	0.087 (8)	27
RS-9	1.262 (6)	0.216 (6)	7.980 (10)	0.762 (7)	0.667 (3)	32
RS-11	2.183 (4)	0.071 (9)	9.376 (9)	0.526 (10)	0.148 (7)	39
RS-13	4.305 (2)	0.187 (7)	14.997 (7)	0.979 (2)	0.361 (4)	22

*Value within parenthesis indicates tolerance ranking
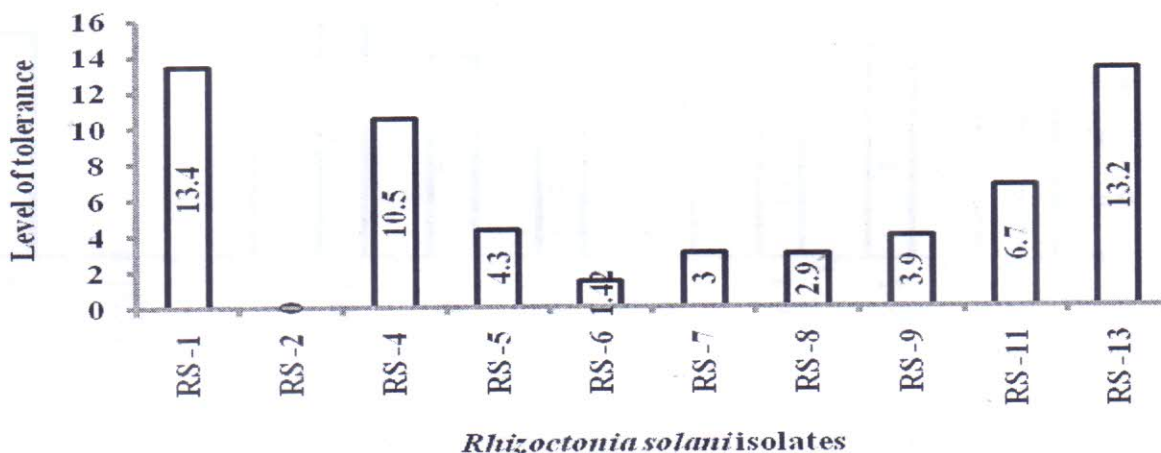

Fig . 1: Tolerance level of *Rhizoctonia solani* isolates to Propiconazole over RS - 2
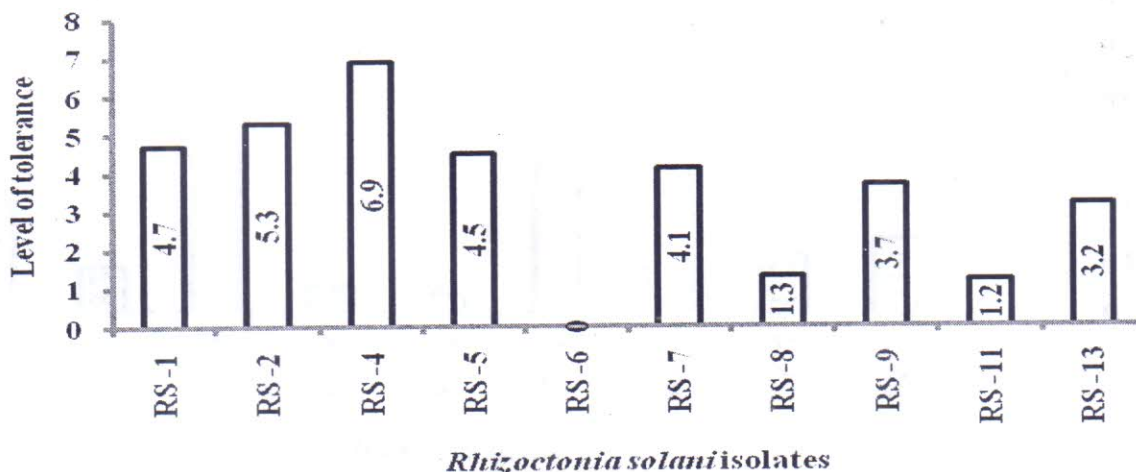

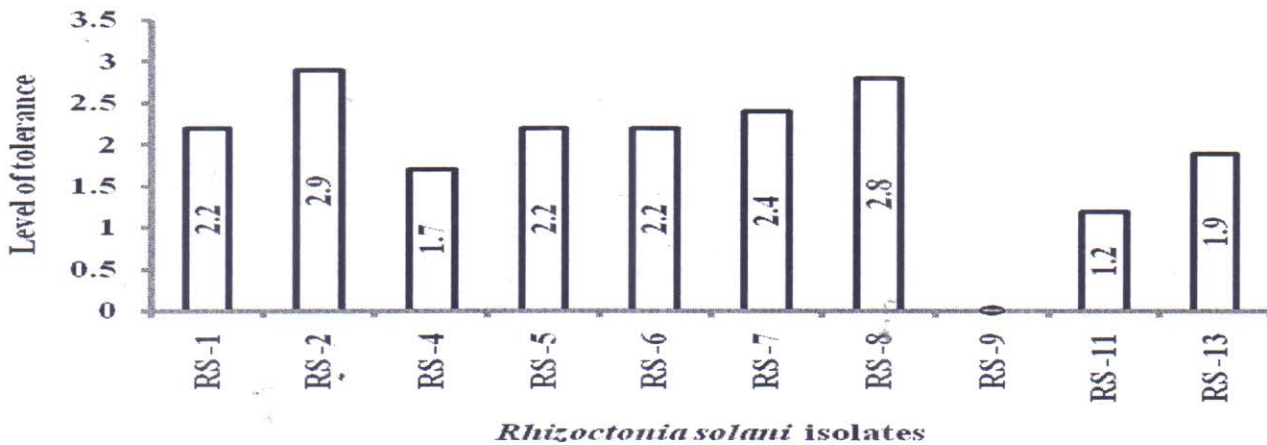Fig . 2: Tolerance level of *Rhizoctonia solani* isolates to Hexaconazole over RS - 6

Fig . 3: Tolerance level of *Rhizoctonia solani* isolates to Mancozeb over RS - 9
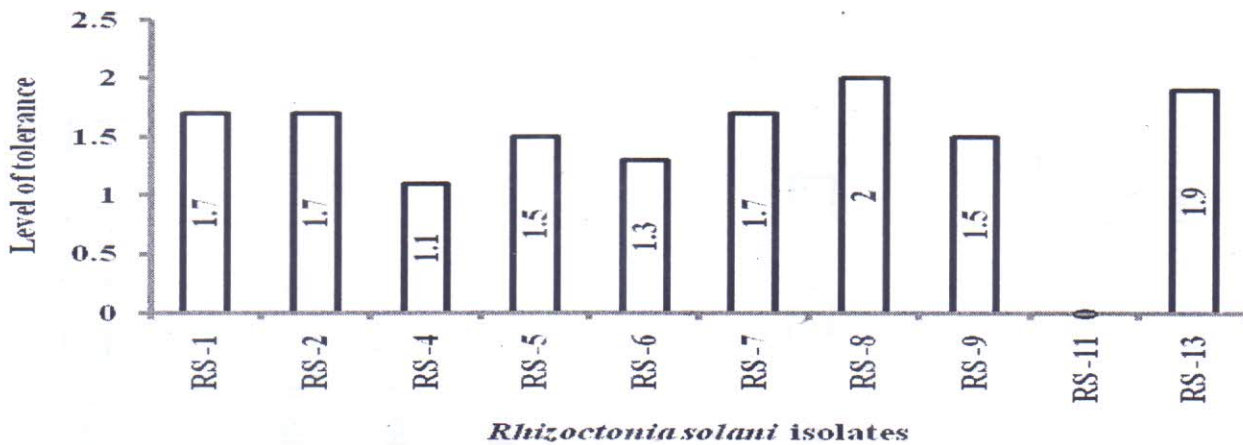

Fig . 4: Tolerance level of *Rhizoctonia solani* isolates to Carbendazim over RS - 11
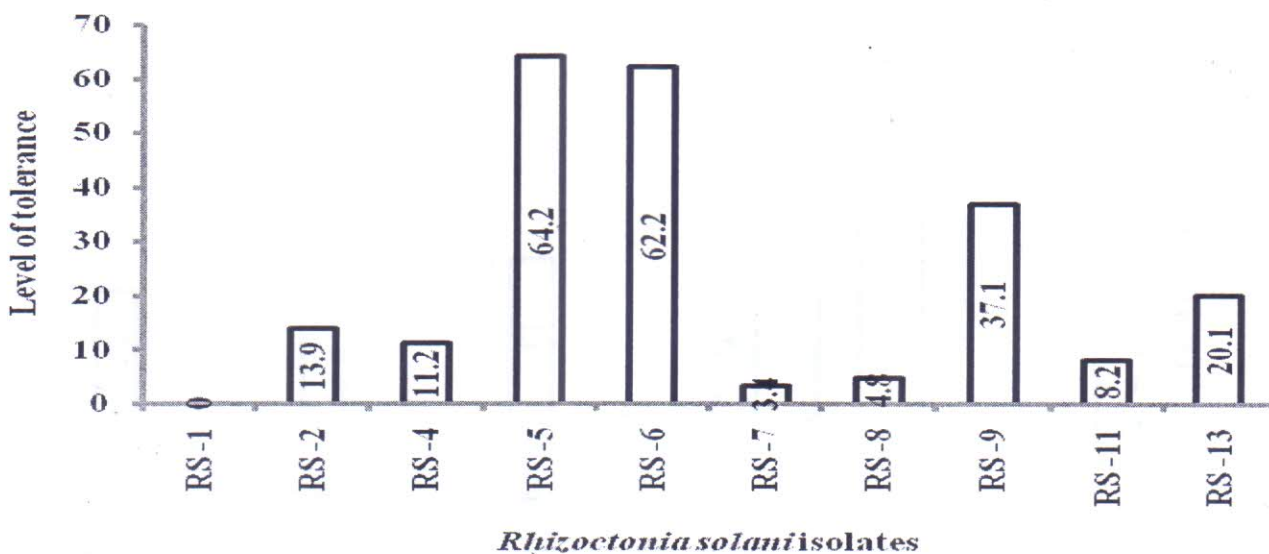

Fig . 5: Tolerance level of *Rhizoctonia solani* isolates toPencycuron over RS - 1

tive to Hexaconazole, RS – 9 showing considerably high tolerance to Pencycuron was sensitive to Mancozeb, RS – 11 exhibiting moderate tolerance to Propiconazole was sensitive to other four fungicides tested. Tolerance level of isolates when judged over fungicides considered, RS - 2, RS - 5 and RS - 13 ranked first and closely followed by RS – 1 whereas RS – 11 ranked last.

It was evident from the results that either a particular isolate of R. solani to five chemically diverse fungicides or all the ten R. solani isolates collected from different geographical locations to a particular fungicide had differential tolerance response as determined on the basis of EC50 values. It was observed that R. solani isolates exhibited 1.4 - 13.4 times tolerance to Propiconazole, 3.4 - 64.2 times to Pencycuron, 1.1 - 2.0 times to Carbendazim, 1.2 - 2.9 times to Mancozeb and 1.2 - 6.9 times to Hexaconazole over the low tolerant/ sensitive one. Though the tolerance property of R. solani isolates is acquired mainly from heterokaryosis resulting from anastomosis ((Taheri et al., 2004) but the quick development of tolerance/resistance along with the reduction in duration of fungicide's effectiveness may sometimes be happened due to unbridled use of fungicides at high doses (Bosch and Gilligan, 2008). The tolerant/ resistant isolate(s) that developed due to continuous exposure of high dose of fungicide is always a great concern and known to have serious economic and environmental consequences. Because, large costs are always involved in developing, testing and releasing novel fungicide molecules as well as in managing disease(s) caused by such isolate(s). In addition, novel fungicides are not always eco-friendly and some may cause environmental degradation. Development of fungicide tolerance in same or different anastomosis groups of R. solani collected from same or different fields, hosts and locations/zones/ geographical areas is known to exist and wide spread. Existence of differential tolerance / sensitivity in R. solani isolates as observed in the present experiment was reported earlier by Ali and Archer (2003) when they tested tolerance of six R. solani isolates to ten fungicides and found the lowest tolerance in Pencycuron followed by Tolclofos methyl, Fludioxionil, Hexaconazole and others. The isolates used for the present experiment were collected from different geographical locations and they differed in their sensitivity to fungicides. This finding corroborated with the observation of Elliott (1999) wherein it was evident that Florida isolates

of Rhizoctonia zeae were more sensitive to Chlorothalonil and the Ohio isolate to Thiram. R. solani isolates even obtained from different hosts may show significant variation in response to fungicides like Pencycuron, Propiconazole, Validamycin, Carbendazim and Carboxin (Thind and Aggarwal, 2008). Besides, isolates of same and different anastomosis groups may vary in their adaptation to a particular fungicide and some adapted isolates may exhibit increased/ reduced/ unaffected aggressiveness. Experiment conducted by Sundar et al. (1993) indicated that isolates like R- 17 (AG7), R – 18(AG- 3) and R – 21 (AG 1 – 1A) adapted more rapidly than other. Adapted isolates were 5 – 11 times less sensitive to Carbendazim than parent isolates. Aggressiveness was increased in adapted R-5 and R – 91 isolates but it was reduced or unaffected in others.

When all ten isolates were tested against five fungicides, it was noted that RS – 1, RS – 4, RS – 2, RS – 8 and RS – 5 though had high tolerance to Propiconazole, Hexaconazole, Mancozeb, Carbendazim and Pencycuron but were sensitive to Pencycuron, Carbendazim, Propiconazole, Pencycuron / Hexaconazole and Carbendazim / Mancozeb respectively. Besides, RS – 6 having high tolerance to Pencycuron was sensitive to Hexaconazole, RS – 9 showing considerably high tolerance to Pencycuron was sensitive to Mancozeb, RS – 11 exhibiting moderate tolerance to Propiconazole was sensitive to other four fungicides tested. So, the areas from where RS – 1, RS – 4, RS – 2, RS – 8, RS – 5, RS – 6 and RS – 9 isolates were collected would remain insensitive to application of Propiconazole, Hexaconazole, Mancozeb, Carbendazim, and Pencycuron respectively. Enough care needs to be taken during selection of appropriate and effective fungicides against these isolates. For the management of these high tolerant isolates, the fungicide(s) against which they were less tolerant/ sensitive could be selected from the cafeteria of five fungicides for alternate or combined but not repeated application. As for example, to control the RS – 1, RS – 13, RS-2, RS-8 and RS-5 isolates, the selection of Pencycuron, Mancozeb / Carbendazim, Propiconazole, Propiconazole/ Hexaconazole/ Pencycuron and Propiconazole/ Carbendazim fungicides could be attempted respectively. Management strategies like dose reduction, fungicide mixtures and alternation of fungicides as proposed by Bosch and Gilligan (2008) to hinder or overcome problem of fungicide resistance

could be followed. Sometimes, the application of two or more fungicides with different modes of action in mixture or in alternation is being widely advocated as a means of delaying or minimizing the risk of the building up of resistance in pathogen population (Brent, 1995).

It can be concluded from the results of experiment that the *R. solani* isolates collected from different geographical areas exhibited differential tolerance to fungicide(s). An isolate obtained from particular area and showing high tolerance to a particular fungicide could not only be managed effectively with application of two or more fungicides against which tolerance is low, in rotation or in mixture but could also be employed as potential and invaluable tool of minimizing the risk of resistance development.

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