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Short communication

Non-target effect of herbicide mixtures on the mycelial growth of *Rhizoctonia solani* Kuhn

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Abstract
Laboratory studies were carried out at Department of Plant Pathology, College of Agriculture,Vellayani to find out the *in vitro* effect of two new post emergence herbicide mixtures *viz*., bispyribac sodium + metamifop 14% SE and penoxsulam + cyhalofop butyl 6% OD on the mycelial growth of soil borne pathogen, *Rhizoctonia solani* causing sheath blight disease in rice. Results revealed that, as the concentration of herbicide mixtures increased a decrease in mycelial growth and an increase in percentage growth inhibition was observed. The lowest concentration of bispyribac sodium + metamifop (100 µl l^{-1}) recorded a colony diameter of 5.27 cm and highest concentration (220 µl l^{-1}) recorded a colony diameter of 0.47 cm with a percentage growth inhibition of 41.48 and 94.81 respectively. Similarly, the lowest concentration of penoxsulam + cyhalofop butyl (230 µl l^{-1}) recorded a mycelial growth of 7.87 cm and the highest concentration recorded a mycelial growth of 0.83 cm diameter with a growth inhibition of 12.61 and 90.74 per cent, respectively. The results indicated that both the herbicide mixtures have immense suppressive effect on the growth of *Rhizoctonia solani*. The inhibitory effect of bispyribac sodium + metamifop 14% SE and penoxsulam + cyhalofop butyl 6% OD on the growth of *Rhizoctonia solani* can be successfully utilized in integrated pest and disease management programme.

Keywords: Bispyribac sodium + metamifop 14% SE, Herbicide mixture, Inhibition, Mycelial growth, Penoxsulam + cyhalofop butyl 6% OD.

Herbicides are known to influence the intensity of plant diseases caused by soil borne pathogens (Katan and Eshel, 1973; Altman and Campbell, 1977). Herbicidal effect on plant diseases has been reported by several workers (Altman and Campbell, 1979; Altman, 1991; Levesque et al., 1992). Herbicides not only control the target weeds but also have non target effect on plant pathogens present in the soil. It may have direct and indirect effect on plant pathogen. Yu et al. (1988) reported that low rate of herbicides stimulates the growth of pathogen under *in vitro* condition. Hence, dose is important in the direct and indirect effect of herbicides on plant diseases. The herbicides influence the plant- pathogen interaction either through their effect on plant or pathogen or on the surrounding soil organisms. Herbicides affect the plant diseases either by altering the virulence of the pathogen or by altering the level of resistance in the host plant (Madhuri et al., 2013). Under *in vitro* condition, herbicides bentazone, benthiocarb, butachlor, nitrofen, pendimethalin, propanil and 2, 4-D sodium salt at 1000 mg l^{-1} completely inhibited the radial mycelial growth of *Rhizoctonia solani* (Das, 1986). Pathak et al. (1996) also reported that 2, 4-D inhibited the growth of *Rhizoctonia solani* under *in vitro* condition. The mycelial growth of *Rhizoconia solani* was inhibited by the herbicides trifluralin and butralin at 5, 50, 100, 500 and 1000 mg l^{-1} (Abdel, 2002). *In vitro* studies revealed that,

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the herbicides butachlor, cimmethyline, glyphosate, bensulfuron and pyrazosulfuron inhibited the mycelial growth of *Rhizoctonia solani* (Shen et al., 2002). Zhu et al. (2002) reported that the mycelial growth of *Rhizoctonia solani* was inhibited by the herbicides, oxyfuorfen, butachlor, acetochlor, cinmethylin and oxadiazon and they also reported that the germination of sclerotia was inhibited by these herbicides at100 mg l^{-1}. The herbicide benthiocarb showed an inhibitory effect on the growth and sclerotia production of *Sclerotium oryzae* under *in vitro* condition (Gupta and Sharma, 2004). Yadav (2006) reported that mycelial growth and sclerotia production of *Rhizoctonia solani* decreased, as the concentration of pyrazosulfuron ethyl in the medium increased from 20 to 70 mg l^{-1}. Gopika et al. (2011) opined that butachlor @ 400 µl l^{-1} was superior in inhibiting the mycelial growth of *Sclerotium oryzae* causing stem rot in rice by 97.1 per cent as compared to oxadiargyl @ 150 µl l^{-1} (27.9 per cent). According to Madhuri and Reddy (2013), oxyfluorfen, alachlor, quizalofop-p-ethyl and 2, 4-D sodium salt were highly effective in inhibiting the growth of *Sclerotium rolfsii* under *in vitro* condition. Pendimethalin, alachlor and quizalofop-p-ethyl recorded 100 per cent growth inhibition of *Rhizoctonia solani* and 2, 4-D sodium salt recorded 100 per cent inhibition in the radial growth of *Fusarium udum*. Rajan et al. (2013) reported that Roundup® at12 ml l^{-1} and paraquat 4 ml l^{-1} inhibited the growth of *Fusarium oxysporumf.* sp. *ciceri* causing wilt in chickpea. Shrivastava (2015) pointed out that the herbicide fluchloralin inhibited the mycelial growth of *Sclerotium rolfsii* causing root rot and collar rot diseases in legumes, crucifers and cucurbits at recommended and double the recommended dose, whereas pendimethalin at recommended and double the recommended dose stimulated the radial growth of *Sclerotium rolfsii*. With this background, the *in vitro* experiments were carried out to find out the non-target effect of two new premix herbicide mixtures, bispyribac sodium + metamifop 14% SE and penoxsulam + cyhalofop butyl 6% OD. Bispyribac sodium + metamifop 14% SE is a combination product of broad spectrum herbicide, bispyribac sodium and a grass effective herbicide metamifop. Similarly, penoxsulam + cyhalofop butyl 6% OD is a combination product of broad spectrum herbicide, penoxsulam and a grass killer cyhalofop butyl.

Laboratory experiments were carried out at Department of Plant pathology, College of Agriculture, Vellayani to study the non-target effect of herbicide mixtures *viz*., bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl on the mycelial growth of *Rhizoctonia solani.* The *in vitro* effect of herbicide mixtures to soil borne pathogen, *Rhizoctonia solani* were determined by poisoned food technique (Zentmeyer, 1955). The experiments were conducted in CRD with 8 treatments (seven different concentrations of herbicide mixtures and a control) and in four replications. The different concentrations of bispyribac sodium + metamifop were 100, 120, 140, 160, 180,200, 220 µl l^{-1}, corresponding to herbicide doses of 50, 60, 70, 80, 90, 100 and 110 g ha^{-1}. The different concentrations of penoxsulam + cyhalofop butyl were 230, 240, 250, 260, 270, 280, 290 µl l^{-1} corresponding to the herbicide doses of 115, 120, 125, 130, 135, 140 and 145 g ha^{-1}.

Stock solution of bispyribac sodium + metamifop (1000 µl l^{-1}) was prepared by dissolving the required quantity of herbicide mixture in sterile water. Fifty ml of 200, 220, 280, 320, 360, 400 and 440 µl l^{-1}, bispyribac sodium + metamifop (double concentration of tested treatments) were prepared in 100 ml conical flask with sterilized water. Fifty ml of double strength potato dextrose agar (PDA) media were prepared in 250 ml conical flasks and sterilized. Fifty ml of double concentration of herbicide were mixed with 50 ml of molten double strength PDA media to get the required concentrations of 100, 120, 140, 160, 180, 200 and 220 µl l^{-1} of the herbicide mixture. After solidification, the plates were inoculated at the centre with 5 mm disc of four day culture of *Rhizoctonia solani*. The control plate was maintained without herbicide. The petri plates were incubated at room temperature. The observations on radial colony diameter in cm were recorded on the day when the full growth of mycelia was observed in control plate i.e., six days after inoculation. Inhibition of radial mycelial growth was measured by the method suggested by Sundar et al. (1995), $\text{Per cent inhibition} = \frac{(X-Y)}{X} \times 100$, where X is the radial growth of mycelia in control plate and Y is the radial growth of mycelia in treated plot. The experiment was repeated for confirmation. Similarly, the *in vitro* effect of penoxsulam + cyhalofop butyl on the mycelial growth of *Rhizoctonia solani* was carried out with the double concentration of the penoxsulam + cyhalofop butyl i.e., 460, 480, 500, 520, 540, 560 and 580 µl l^{-1} and double dextrose potato dextrose agar media by adopting the same procedure as described above as in the case of bispyribac sodium + metamifop. The experiment was repeated for confirmation.

Perusal of data on the *in vitro* sensitivity of bispyribac sodium + metamifop to soil borne pathogen, *Rhizoctonia* revealed that, with an increase in the concentration of the herbicide, a significant reduction in the mycelial growth of *Rhizoctonia solani* was observed (Table 1).

All the tested concentrations of herbicide mixture, bispyribac sodium + metamifop significantly reduced the mycelial growth of the pathogen. The lowest concentration of 100 µl l^{-1} recorded the maximum colony diameter (5.27 cm) with a growth inhibition of 41.48 per cent, which was followed by 120 µl l^{-1} concentrations and the same was statistically on par with 140 µl l^{-1} in reducing the colony diameter. The percentage inhibitions in mycelial growth in these treatments were 56.30 and 57.41, respectively. Concentration of herbicide mixture of 160 µl l^{-1} recorded a colony diameter (2.77 cm) which was followed by other concentrations of 180, 200 and 220 µl l^{-1}. All these treatments were statistically different from each other. These treatments recorded the colony diameter of 2.77, 2.10, 1.57 and 0.47 cm, respectively with a growth inhibition of 69.26, 76.67, 82.59 and 94.81 per cent, respectively. Critical appraisal of data revealed that, more than 50 per cent inhibition in mycelial growth was observed at concentrations above 120 µl l^{-1}. The maximum growth inhibition (94.81 per cent) was observed in the highest tested concentration (220 µl l^{-1}) corresponding to the herbicide dose of 110 g ha^{-1}. The variation in the inhibitory effect on *Rhizoctonia solani* observed among the treatments might be due to the difference in the concentration of the herbicide. This is in line with the observations made by Bollen (1961), Hattori (1973) and Sebiomo et al. (2011). The inhibitory effect of bispyribac sodium + metamifop on the growth of

Table 1. In vitro effect of bispyribac sodium + metamifop on the mycelial growth of *Rhizoctonia solani*

Bispyribac sodium + metamifop dose tested, µl l^{-1}	Growth of *Rhizoctonia solani*: Colony diameter, cm at 6 DAI		Percentage Inhibition	
0 (Control)	9.00	(3.08)		0
100	5.27	(2.40)	41.48	(40.09)
120	3.93	(2.10)	56.30	(48.62)
140	3.83	(2.08)	57.41	(49.27)
160	2.77	(1.81)	69.26	(56.33)
180	2.10	(1.61)	76.67	(61.33)
200	1.57	(1.43)	82.59	(65.35)
220	0.47	(0.98)	94.81	(76.85)
SEm (±)		0.022		0.619
CD (0.05)		0.066		1.880

DAI - Days after incubation, values in parentheses are transformed values (colony diameter-square root transformation $\sqrt{(x+0.5)}$, per cent inhibition - arcsine transformation).

Table 2. In vitro effect of penoxsulam + cyhalofop butyl on the mycelial growth of *Rhizoctonia solan*i

Penoxsulam + cyhalofop butyl dose tested , µl l^{-1}	Growth of *Rhizoctonia solani*			
	Colony diameter, cm at 6	DAI	Percentage	Inhibition
0 (Control)	9.00	(3.08)		0
230	7.87	(2.89)	12.61	(20.07)
240	5.20	(2.39)	42.22	(40.52)
250	4.27	(2.18)	52.59	(46.49)
260	3.27	(1.94)	63.70	(52.95)
270	2.07	(1.60)	77.04	(61.37)
280	1.23	(1.31)	86.30	(68.28)
290	0.83	(1.15)	90.74	(72.31)
SEm (±)		0.032		1.578
CD (0.05)		0.095		4.732

DAI - Days after incubation, values in parentheses are transformed values (colony diameter-square root transformation $\sqrt{(x+0.5)}$, per cent inhibition - arcsine transformation)

Rhizoctonia solani along with their effectiveness in weed control can be exploited under integrated pest and disease management programme. Madhuri and Reddy (2013) reported that pendimethalin, alachlor and quizalofop-p-ethyl recorded 100 per cent growth inhibition of *Rhizoctonia solani.* Similarly, Das (1986) and Harikrishnan and Yang (2001) reported that pendimethalin significantly reduced the mycelial growth of *Rhizoctonia solani*.

Similar to bipsyribac sodium + metamifop, with an increase in the concentration of penoxsulam + cyhalofop butyl, a corresponding reduction in the mycelial growth of *Rhizoctonia solani* was observed (Table 2). All the concentrations of penoxsulam + cyhalofop butyl significantly inhibited the radial growth of mycelia and the percentage growth inhibition ranged from 12.61 to 90.74. Critical appraisal of the data revealed that more than 50 per cent inhibition in the mycelial growth of *Rhizoctonia* was observed in concentration of 250 µl l^{-1}. The maximum inhibition in the growth of *Rhizoctonia* (90.74 per cent) was observed in 290 µl l^{-1} with a colony diameter of 0.83 cm. The lowest inhibition (12.61 per cent) was observed in 220 µl l^{-1} with the highest radial mycelial growth (7.87 cm) excluding control.

The concentrations of penoxsulam + cyhalofop butyl, 240, 250, 260 and 270 µl l^{-1} which corresponds to the herbicide doses of 120, 125, 130 and 135 g ha^{-1}registered an inhibition in the mycelial growth of *Rhizoctonia solani* by 42.22, 52.59, 63.70 and 77.04 per cent. The difference in the concentrations of the herbicide has resulted in variation in mycelial growth. Sebiomo et al. (2011) reported that the effect of herbicides on soil fungi varied among herbicides depending on the application rates. The above findings throw light on the additional benefits that can be derived through the application of herbicide mixture, penoxsulam + cyhalofop butyl. In addition to its powerful effect in reducing the weed density in direct seeded rice, the *in vitro* results have shown that it has immense suppressive effect on the growth of dreaded soil borne pathogen, which cause sheath blight disease in rice. Due to the inhibitory effect on the growth of *Rhizoctonia solani*, the herbicide mixture, penoxsulam + cyhalofop butyl can be successfully utilized in integrated pest and disease management programme. Several researchers have reported the effectiveness of herbicides in inhibiting the growth of *Rhizoctonia solani* under *in vitro* condition. Under *in vitro* condition, butachlor @ 400 µl l^{-1} was found superior in inhibiting the mycelial growth of *Sclerotium oryzae* by 97.1 per cent as compared to oxadiargyl @ 150 µl l^{-1} (27.9 per cent) Gopika et al. (2011). Abdel (2002) has well documented the effectiveness of herbicides trifluralin and butralin in inhibiting the growth of *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Yadav (2006) also reported that mycelial growth and sclerotia production of *Rhizoctonia solani* decreased, as the concentration of pyrazosulfuron ethyl in the medium increased from 20 to 70 mg l^{-1} under *in vitro* condition.

The results revealed the inhibitory effect of herbicide mixtures on the radial mycelial growth of *Rhizoctonia solani*. As the concentration of the herbicide increased, a decrease in radial mycelial growth of the fungi was observed in both the herbicide mixtures. The lowest tested concentration ofbispyrbac sodium + metamifop (100 µl l^{-1}) recorded the maximum colony diameter of 5.27 cm with a growth inhibition of 41.48 per cent and the highest tested concentration (220 µl l^{-1}) recorded the colony diameter of 0.47 cm with a growth inhibition of 94.81 per cent. The doses of penoxsulam + cyhalofop butyl *viz*., 120, 125, 130 and 135 g ha^{-1} corresponding to laboratory doses of 230, 240, 260 and 270 µl l^{-1} registered an inhibition in the mycelial growth of *Rhizoctonia solani* by 42.22, 52.59, 63.70 and 77.04 per cent, respectively. The highest tested dose (290 µl l^{-1}) inhibited the radial growth of *Rhizoctonia solani* by 90.74 per cent. The above findings throw light on the additional benefits of disease suppression that can be derived through the application of herbicide mixtures, penoxsulam + cyhalofop butyl 6% OD and bispyribac sodium + metamifop 14% SE. The inhibitory effect of herbicide mixtures on the growth of *Rhizoctonia solani* along with their effectiveness in weed control can be exploited under integrated pest and disease management programmes.

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