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Compatibility of biocontrol agents and N fixing organisms with post emergence pre-mix herbicide-bispyribac sodium + metamifop 14 % SE

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Abstract: The experiments were conducted *in vitro* in the Agricultural Microbiology laboratory at College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India to evaluate the compatibility of biocontrol agents *Pseudomonas fluorescens* and *Tricoderma viride* and N fixing organisms *Azospirillum lipoferum* and *Azotobacter chroococcum* to bispyribac sodium + metamifop 14 % SE, a new broad spectrum post emergence pre-mix herbicide mixture used for weed control in rice. The experiments were conducted in completely randomized block design with seven different concentrations of herbicide *viz*., 100, 120, 140, 160, 180, 200 and 220 µL L^{-1} corresponding to field doses of 50, 60, 70, 80 90, 100 and 110 g ha^{-1} and a control (0 µL L^{-1}). All the tested concentrations of the herbicide mixture were highly compatible with *P. fluorescens*, *A. lipoferum* and *A. chroococcum*. The radial colony diameter of *T. viride* was significantly influenced by different concentrations of the herbicide. The field dose of bisspyribac sodium + metamifop up to 90 g ha^{-1} (180 µL L^{-1}) is harmless to *T. viride*, since it recorded a growth inhibition of only 22.96 per cent but higher doses (100 and 110 g ha^{-1}) which recorded a growth inhibition of 31.48 and 37.04 per cent respectively were slightly harmful to the antagonistic fungus. The compatibility results revealed the possibility of using bispyribac sodium + metamifop for weed control at recommended doses (70, 80 or 90 g ha^{-1}) under bio intensive disease management programme involving *P. fluorescens* / *T. viride* and nutrient management programme involving *A. liopferum* / *A chroococcum*.

Keywords: Bio control agents, Bispyribac sodium + metamifop, Compatibility, Herbicide mixture, N fixing organisms

INTRODUCTION

In sustainable agriculture, soil is viewed as a fragile and living medium that must be protected and nurtured to ensure its long term productivity and stability. Sustainable agriculture is gaining popularity today due to lesser environmental pollution as it relays on the use of the chemical inputs *viz*., fertilizers, pesticides, fungicides and herbicides, in an economically viable and ecofriendly manner and promote the use of bio-control agents and bio-fertilizers to the possible extent. Seed treatment with bio control agents *viz*., *P. fluorescens* and *T. viride* is the most widely used practice among the farmers to reduce the incidence of seed and soil borne pathogens and enhance the productivity in rice. These biocontrol agents are highly host specific, virulent, self-perpetuating and genetically stable (Gangwar, 2013) and influence the plant growth by producing secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones. *P. fluorescens* is effective against various fungal diseases *viz*., sheath blight caused by *Rhizoctonia solani* (Krishnakumari, 2016), blast caused by *Pyricularia oryzae* (Shyamala and Shivakumar, 2012) and sheath rot caused by *Sarocladium oryzae* (Saravanakumar *et al*., 2009) and bacterial blight caused by *Xanthomonas oryzae pv oryzae* (Gangwar and Sinha, 2012). *T. viride* is known for its mycoparasitic and antagonistic mechanism for the control of fungal disease in rice *viz*., brown leaf spot caused by *Bipolaris oryzae* and sheath blight caused by *Rhizoctonia solani* (Biswas and Datta, 2013). The combined use of biocontrol agents and pesticides results in synergistic or additive effects in the control of soil borne pathogens (Clarkson *et al*., 2006). So, for the successful adoption of integrated pest management programme in rice production, it is necessary to screen the compatibility of biocontrol agents with the herbicides. Several studies have been conducted *in vitro,* to evaluate the negative effects of insecticides and fungicides on the growth and development of biocontrol agents (Hirose *et al*., 2001; Neves *et al*., 2001; Silva

and Neves, 2005), however the studies regarding the side effects of different group of herbicides on the growth and development of bio control agents are meagre (Santoro *et al*., 2014).

The use of herbicides has become an integral part of agriculture to control the weeds, which cause severe economic loss to the farmers. Herbicides not only have adverse effect on the plant growth, but also influence the plant growth by the additive and synergistic interaction between plant growth promoting bacteria (Brock, 1975). Biofertilizers are the environment friendly alternative to the chemical fertilizers for increasing the soil fertility and crop productivity without causing any harmful environmental effects (Hermosa e*t al*., 2012). *Azospirillum* and *Azotobacter* are the two potential N bio-fertilizers for rice cultivation and have the ability to fix about 20 to 25 kg N ha^{-1} (Kizilkaya, 2009; Sattar *et al*., 2008). They not only fix atmospheric N but also stimulate the plant growth by producing growth stimulating hormones such as auxins, cytokinins and gibberellic acid (Poureidi *et al*., 2015). These bio-inoculants are exposed to herbicides either at the time of planting or later in the season (Jeenie *et al*., 2011). Screening the compatibility of *Azospirillum* and *Azotobacter* with herbicides will pave the way for the combined use of herbicides and these N fixing organisms. Some herbicides used in agriculture may have negative effect on the growth of these organisms due to the difference in the mode of action, concentration of the herbicide or chemical group. Evaluation of new herbicides for their toxicity to bio fertilizer organisms will enable the rice growers to select the compatible ones. Hence, the present study was undertaken with an objective to assess the compatibility of *P. fluorescens*, *T. viride*, *A. lipoferum* and *A. chroococcum* with the new pre-mix herbicide mixture, bispyribac sodium + metamifop 14 % SE, a combination product of broad spectrum herbicide, bispyribac sodium and grass effective herbicide, metamifop.

MATERIALS AND METHODS

The experiments were conducted *in vitro*, to study the relative compatibility of *P. fluorescens*, *T. viride*, *A. lipoferum* and *A. chroococcum* with different doses of herbicide mixture bispyribac sodium + metamifop. The design of the study was completely randomized block design with eight treatments comprising of seven different concentrations of herbicide, bispyribac sodium + metamifop and a control. The different herbicide concentrations were 100, 120, 140, 160, 180, 200 and 220 µL L^{-1}corresponding to herbicide doses of 50, 60, 70, 80, 90, 100 and 110 g ha^{-1} and control (0 µL L^{-1}). The experiments were carried out in the Agricultural Microbiology laboratory, College of Agriculture, Vellayani, Kerala, India.

The *in vitro* sensitivity of *T. viride* to bispyribac sodium + metamifop was determined by poison food technique, the method suggested by Zentmeyer, 1955. The experiment was repeated for confirmation. In this method, 1000 ppm stock solution of bispyribac sodium + metamifop was prepared by dissolving the required quantity of herbicide mixture in sterile water. Fifty mL double strength potato dextrose agar (PDA) media were prepared in 250 mL conical flasks and sterilized. In 100 mL, conical flasks 50 mL of respective double concentration of herbicides were prepared with sterilized water. These 50 mL double concentrations of herbicide were mixed with 50 mL molten double strength PDA media to get the required concentrations of the herbicide mixture. After solidification, the plates were inoculated at the center with 5 mm disc of *T. viride* culture of four days' growth. The control plate was maintained without herbicide. The petri plates were incubated at room temperature. The observations on radial colony diameter were recorded on the day when the full growth of mycelia was observed in control plate *i.e.,* on 6 days after incubation (DAI). Inhibition of radial mycelial growth was measured by the method suggested by Sundar *et al*. (1995) and the percentage inhibition was worked out using the formula:

$I = (X-Y/X) \times 100$

Where I is the per cent inhibition in mycelia growth

X is the radial growth of mycelia in control plate

Y is the radial growth of mycelia in treated plot.

The compatibility of *P. fluorescens*, *A. lipoferum* and *A. chroococcum* with the herbicide mixture, bispyribac sodium + metamifop was determined by disc diffusion method (Bauer *et al*., 1966). In this method, 20 mL of sterilized King's B medium, NFb (Nitrogen free bromothymol blue) medium and Jensen medium were poured into 90 mm sterile petri plates; after solidification the petri plates were stored for 24 h to ensure the sterility. The petri plates containing NFb medium were swabbed with broth suspension of *A. lipoferum*, Jensen medium with *A. chroococcum* and King's B medium with *P. fluorescens* having four days' growth. Sterile filter paper disc of 6 mm dipped in respective concentrations of herbicide were placed at the centre of the petri plate. Sterile filter paper disc dipped in sterile water served as the control. The petri plates were sealed and kept for 3 days' incubation at room temperature. Four replications were maintained for each treatment. The observations on inhibition zone in mm were recorded at 3 DAI and the growth was visually growth was categorized as positive culture growth (+) around the disc and inhibited culture growth (-) around the disc. The experiment was repeated for confirmation.

The data were subjected to analysis of variance (ANOVA) and critical difference between the treatments means were compared at 5 per cent probability level.

RESULTS AND DISCUSSION

The *in vitro* sensitivity of *T. viride* to bispyribac sodium + metamifop at tested concentrations is presented

in Table 1. Bispyribac sodium + metamifop significantly influenced the colony diameter and percentage growth inhibition. The variation in mycelial growth of *T. viride* was observed in the medium poisoned with tested concentrations of bispyribac sodium + metamifop *viz*., 100, 120, 140, 60, 180, 200 and 220 µL L^{-1} corresponding to herbicide doses of 50, 60, 70, 80, 90, 100 and 110 g ha^{-1}. With increase in the concentration of herbicide in the medium a decrease in colony diameter and increase in percentage growth inhibition of *T. viride* was observed at 6 DAI. The lowest concentration (100 µL L^{-1}) recorded the highest colony diameter (8.53 cm) and the highest tested concentration recorded the lowest colony diameter (5.67 cm). The growth inhibition registered by *T. viride*, exposed to bispyribac sodium + metamifop @ 100 µL L^{-1} to 220 µL L^{-1} ranged from 5.19 to 37.04 per cent. The field-tested doses of 60, 70, 80 and 90 g ha^{-1} corresponding to laboratory doses of 120, 140, 160 and 180 µL L^{-1} recorded the growth inhibition of 8.15 to 22.96 per cent only. According to International Organization for Biological Control (IOBC) toxicity classification scheme (Sterk *et al*., 2002), the tested chemical that produce < 25 per cent inhibition falls in Class I toxicity category and is considered harmless; if it produces a growth inhibition between 25 to 50 per cent, the tested chemical falls in class II toxicity category and is slightly harmful to the beneficial fungi tested. Hence, the laboratory doses *viz*., 120, 140, 160 and 180 µL L^{-1} corresponding to field doses of 60, 70, 80 and 90 g ha^{-1} is harmless and safe to *T. viride*, whereas, the highest tested concentrations of 200 and 220 µL L^{-1}, corresponding to laboratory doses of 100 and 110 g ha^{-1}, registered the mycelial growth inhibition of 31.48 and 37.04 per cent respectively and were slightly harmful to *T.viride*. These results indicated that *T. viride* is sensitive to bispyribac sodium + metamifop, at higher concentrations (more than 180 µL L^{-1}). Santoro *et al*. (2014) reported that, the herbicides, 2, 4-D, clomazone and imazapyr were compatible with *T. atroviride*. Compatibility of *Tricoderma sp*. to herbicides was also reported by several researchers. Saxena *et al*. (2014) reported that 2, 4-D ethyl ester, pretilachlor, aniliofos, alachlor, butachlor, fluchloralin and pendimethalin were found compatible with the test antagonist *T. harzianum* (PBT23) even at higher concentration (250 µL mL^{-1}). An increase in colony forming unit of *T. viride* was observed with 50 µL L^{-1} butachlor (Rao and Divakar, 2002).

In vitro sensitivity results revealed that bispyribac sodium + metamifop at different tested concentrations *viz*., 100, 120, 140, 160, 180, 200 and 220 µL L^{-1} corresponding to the field doses of 50, 60, 70, 80, 90, 100 and 120 g ha^{-1} did not exert any inhibition on the growth of *P. fluorescens*, *A. lipoferum* and *A. chroococcum* (Table 2). No inhibition zone was observed around the sterile disc impregnated with herbicide in tested concentrations (zero to 220 µL L^{-1}). Positive culture growth was observed around the disc and was very similar to that of control plate. These results indicated that *P. fluorescens*, *A. lipoferum* and *A. chroococcum w*ere not sensitive to bispyribac sodium + metamifop at tested doses *viz*., 100, 120, 140, 160, 180, 200 and 220 µL L^{-1} corresponding field doses of 50, 60, 70, 80 and 90 100 and 120 g ha^{-1}. It appears that even at higher dose of 110 g ha^{-1}, bispyribac sodium + metamifop had no adverse impact on the growth of *P. fluorescens, A. lipoferum* and *A. chroococcum*. Compatibility of *P. fluorescens* with herbicides butachlor and pendimethalin even at higher doses of 1000 and 2000 µL $L^{-1}$was reported by Gangwar (2013). Surendran *et al*. (2012) opined that *P. fluorescens* was highly compatible with 2, 4-D sodium salt, pyrazosulfuron ethyl, cyhalofop butyl, penoxsulam and bispyribac sodium at recommended field doses of 3.125 g L^{-1}, 3.2 mL L^{-1}, 0.7 g L^{-1}, 0.3 mL L^{-1} and 1 mL L^{-1}respectively. Studies on the effect of 2, 4-D sodium salt on the growth and nitrogenase activity of *A. brazilense* re-

Table 1. *In vitro* effect of herbicide, bispyribac sodium + metamifop on the radial growth of *T. viride*.

Bispyribac sodium + metamifop concentrations (µL L^{-1})	**Growth of *T.viride***	
	Colony diameter (cm) at 6 DAI	**Per cent growth Inhibition**
0 (Control)	9.00	0
100	8.53	13.15 (5.19)
120	8.27	16.44 (8.14)
140	7.57	23.52 (15.93)
160	7.50	24.10 (16.67)
180	6.93	28.63 (22.96)
200	6.17	34.11 (31.48)
220	5.67	37.49 (37.04)
SEm (±)	0.093	0.841
CD (0.05)	0.271	2.550

DAI- Days after incubation, values in parentheses are original values- data are subjected to Arc sine transformation

Table 2. *Compatibility* of *P. fluorescens*, *A. lipoferum* and *A. chroococcum* with Bispyribac sodium + metamifop.

Bispyribac sodium + metamifop concentrations (µL L^{-1})	Growth of *P. fluorescens* at 3 DAI	Growth of *lipoferum* at 3 DAI	Growth of *chroococcum* at 3 DAI
0 (Control)	+	+	+
100	+	+	+
120	+	+	+
140	+	+	+
160	+	+	+
180	+	+	+
200	+	+	+
220	+	+	+

+ : Positive culture growth around the sterile disc, DAI- Days after incubation

vealed that the herbicide had no negative effect at 100, 200 and 300 µg mL^{-1} (Martinez-Toledo *et al*., 1990). Similarly, Gadkari and Klingmuller (1988) observed that the herbicides metamitron at 35 and 75µg mL^{-1} and ethiozin at 20 µg mL^{-1} did not have any inhibitory effect on the growth of *A. liopferum* and *A. brazilense*. Mrkovacki *et al*. (2002) pointed out that the growth of three tested strains of *A. chroococcum* was unaffected even at 10 times recommended dose of herbicides *viz*., cycloate and chloridazon. The results obtained in the present study could be exploited for the combined application of these organisms along with the herbicide mixture, bispyribac sodium + metamifop at the tested field doses, to enhance their population in the soil, as these bacteria are important for sustaining the productivity of soil.

Conclusion

It can be concluded from the study that, post emergence herbicide mixture, bispyribac sodium + metamifop is highly compatible to beneficial bacteria *P. fluorescence*, *A. lipoferum* and *A chroococcum* even at higher dose of 110 g ha^{-1}. The study also revealed the compatibility of bispyribac sodium + metamifop to antagonistic fungi, *T. viride*. Bipyribac sodium + metamifop is harmless and safe to *T. viride* at doses ranging from 60 to 90 g ha^{-1}, since it recorded a growth inhibition of 8.15 to 22.95 per cent and falls in Class I toxicity category. The highest tested doses (100 and 110 g ha^{-1}) recorded a growth inhibition of 31.48 and 37.04 per cent respectively and fall in Class II toxicity category and are slightly harmful to *T. viride*. The compatibility results established the possibility of using bispyribac sodium + metamifop at recommended doses (70, 80 or 90 g ha^{-1}) for weed control with *P. fluorescens* / *T. viride* for the effective management of bacterial and fungal diseases and *A. lipoferum* / *A. chroococcum* for reducing the use of N fertilizers in rice in single application with environmental safety.

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