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Nontarget effects of various Herbicides on biocontrol agent *Trichoderma spp*. and pathogen *Sclerotium rolfsii*

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Abstract

Trichoderma spp. possesses most of the mechanisms responsible for biological control and plant growth promotion. However, the activity of *Trichoderma* spp. in soil is highly influenced by several factors. Among all the pesticides used in agriculture, herbicides stand first and are widely applied to soil as pre-emergence to manage a variety of weeds. However, indiscriminate use of herbicides has raised several environmental concerns. Deleterious effects of these herbicides may affect the population and activity of biocontrol agent *Trichoderma* that is primarily soil inhabitant as well as may also affect the population of *Sclerotium rolfsii*. Thus, this study was carried out to find out the effect of various herbicides on radial growth of *Trichoderma* sp. and *Sclerotium rolfsii*.

Nine different pre-emergence *i.e.* alachlor, butachlor, anilophs, metribuzin, pretilachlor Metolachlor, pendimethalin, oxadiargyl, oxyflurfen and the preplant incorporation (fluchloralin) herbicides were evaluated for their effect on radial growth of *Trichoderma* sp. and *S. rolfsii*. In three different concentrations *i.e*. 0.5 X (half of recommended). Periodic observations were recorded on the radial growth with a view to find out the action of herbicides against *Trichoderma* sp. and *S. rolfsii*.

Radial growth of *S. rolfsii* was stimulated by pendimenthalin at X and 2X and Metolachlor at 0.5X concentrations under *in vitro* conditions. Herbicide oxadiargyl inhibited the radial growth of bio-agent and its higher concentrations were more inhibitory than the lower one. On the basis of visual observations, flluchloralin X and metribuzin 2X slightly reduced the sporulation of bioagent whereas it was slightly stimulated by pendimethalin X.

Herbicide alachlor stimulated the total mycelia growth of bioagent and reduced the mycelia growth of pathogen. This differential action of alachlor could be a promising achievement for combined management of weeds and soil borne crop diseases.

The fungal genus *Trichoderma* has gained immense importance since last few decades due to its biological control potential against several plant pathogens[10,11,14]. By recognizing the importance of this fungus as biocontrol agent against several plant pathogens[16] it is sold under different trade names, for example: *Trichoderma viride* as Ecofit and Bhasderma and *Trichoderma harzianum* as F-stop, Trichodex, Binab-T, M.T.R.-35, T.39 etc.

Sclerotium rolfsii is an important plant pathogen, which has an extensive host range. At least 500 species in 100 families are susceptible. The most common hosts are the legumes, crucifers, and cucurbits. The important plant diseases caused by *S. rolfsii*, include root rot of beet, root and collar rot of chickpea, collar and bean rot disease of sword bean[8], rot of chestnut –*Trapa bispinosa*[19], collar rot of safflower[20].

S. rolfsii grows, survives, and attacks plants at or near the soil line. Before the pathogen penetrates host tissue it produces a considerable mass of mycelium on the plant surface, a process that can take two to ten days. Penetration of host tissue occurs when the pathogen produces an enzyme, which deteriorates the host's outer cell layer. This results in tissue decay and further production of mycelium.

Trichoderma spp. are known mycoparasite of a number of plant pathogens[11,15]. *T. harzianum* colonizes *S. rolfsii* hyphae, disrupts mycelial growth, and kills the organism[6,13]. *T. viride* has been shown to provide good control, especially when used in combination with an herbicide or pesticide. *Trichoderma virens* have been shown to rapidly degrade *S. rolfsii* in soil.

Trichoderma spp. possesses most of the mechanisms responsible for biological control and plant growth promotion. However, the activity of *Trichoderma* spp. in soil is highly influenced by several factors. Among all the pesticides used in agriculture, herbicides stand first and are widely applied to soil as pre-emergence to manage a variety of weeds[2]. However, indiscriminate use of herbicides has raised several environmental concerns. Deleterious effects of these herbicides may affect the population and activity of biocontrol agent *Trichoderma* that is primarily soil inhabitant as well as may also affect the population of *Sclerotium rolfsii.* Thus this study was carried out to find out the effect of various herbicides on radial growth of *Trichoderma* spp. and *Sclerotium rolfsii.*

Effect of herbicides on radial growth of *Sclerotium rolfsii*:

Nine different pre-emergence and one pre-plant incorporation (fluchloralin) herbicides were evaluated for their effect on radial growth of *S. rolfsii*. Desired quantity of herbicides (maintained through serial dilution as mentioned in Table (1) were poured into sterilized Petri plates for 0.5X, X and 2X concentrations of each herbicide. Thereafter, freshly prepared sterilized and cooled Czapek Dox Agar (CDA) was poured into each petriplate. Five replications were maintained for each treatment. After solidification plates were inoculated with 8mm. disc of *S. rolfsii* and then incubated at 28±1°C temperature. Periodic observations

were recorded on the radial growth of *S. rolfsii*.

Effect of herbicides on radial growth of *Trichoderma* spp.:

All ten different herbicides were evaluated for their effect on radial growth of *Trichoderma* sp. Desired quantities of herbicides (maintained through serial dilution) were poured into sterilized Petri plates for 0.5X, X and 2X concentration of each herbicide. Thereafter, freshly prepared sterilized and cooled Czapek Dox Agar was poured into each Petriplate and shaken thoroughly. Five replications were maintained for each treatment. After solidification plates were inoculated with 8mm. disc of *Trichoderma* sp. and then incubated at 28±1°C temperature. Periodic observations were recorded on the radial growth of *Trichoderma* sp.

Data in the Table (2) clearly shows that anilophos, metribuzin and pretilachlor, on an average stimulated the radial growth of *S. rolfsii* at all the concentrations except anilophos at 2X, which has stimulated the radial growth at initial intervals but later it was at par with control. Butachlor at 0.5X slightly stimulated the radial growth at initial intervals but later it was at par with control. Whereas, there was little bit inhibition in radial growth with X and 2X concentration of butachlor. Alachlor was at par with control at all the concentrations.

Metolachlor, pendimethalin, oxadiargyl and oxyfluorfen showed marked inhibition in radial growth of *S. rolfsii*. However, drastic reduction in the radial growth of *S. rolfsii* was recorded with all the concentrations of fluchloralin. This may serve as a promising herbicide for heavily infested fields of *S. rolfsii*.

Table-1. Concentrations (ppm) of different herbicides used for the experiments

S.no.	Herbicide	Recommended rate (X) g.a.i. /ha	Commercial formulation g. /ha	ppm (X)
1.	Butachlor	1000	2000	1.0
2.	Alachlor	2000	4000	2.0
3.	Metolachlor	1500	3000	1.5
4.	Fluchloralin	1000	2000	1.0
5.	Anilophos	400	1200	0.6
6.	Metribuzin	525	750	0.375
7.	Pendimethalin	1000	3000	1.5
8.	Oxyfluorfen	125	500	0.25
9.	Oxadiargyl	90	1500	0.75
10.	Pretilachlor	500	1000	0.5

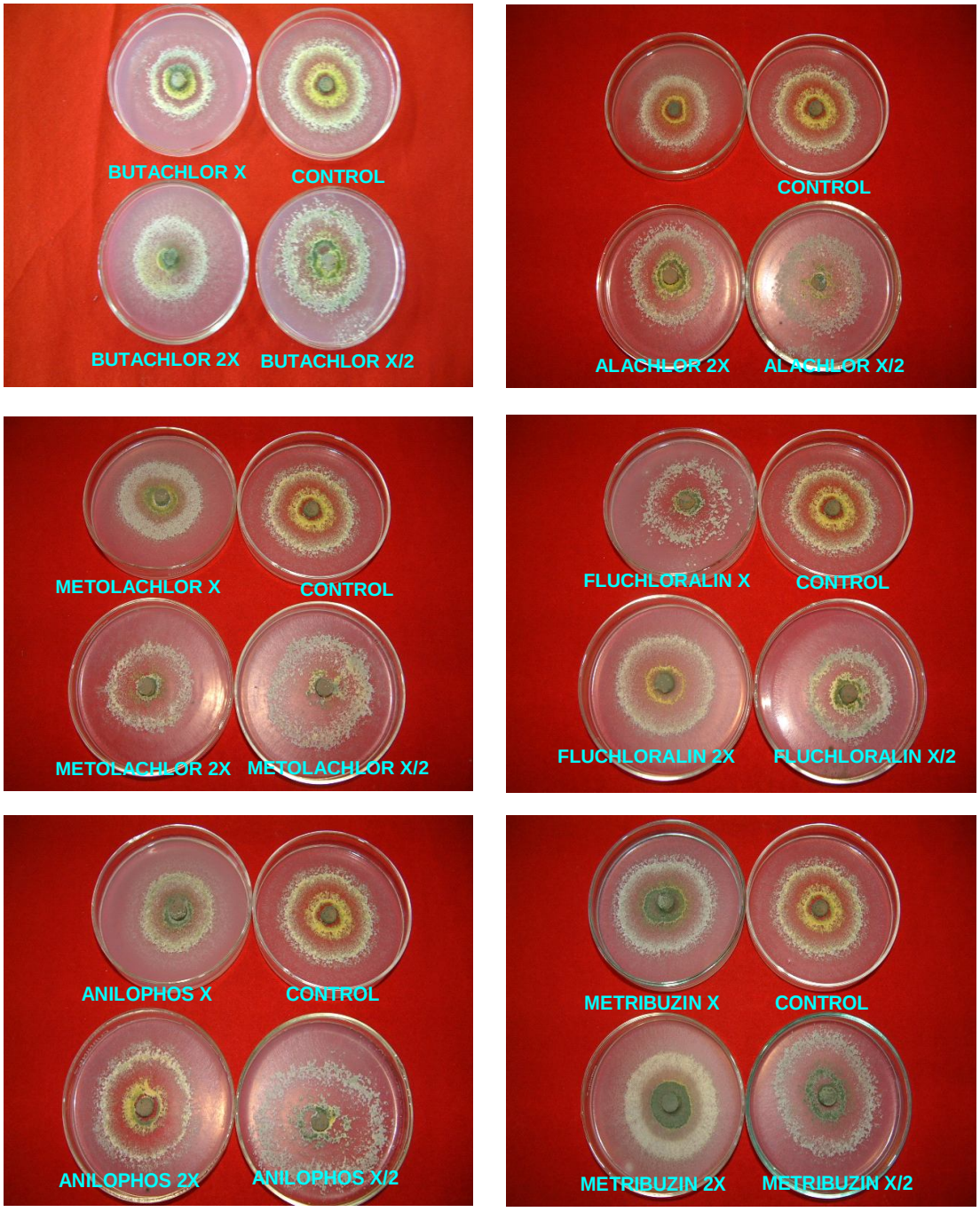
Table-2. Effect of different herbicides on radial growth of *Sclerotium rolfsii*

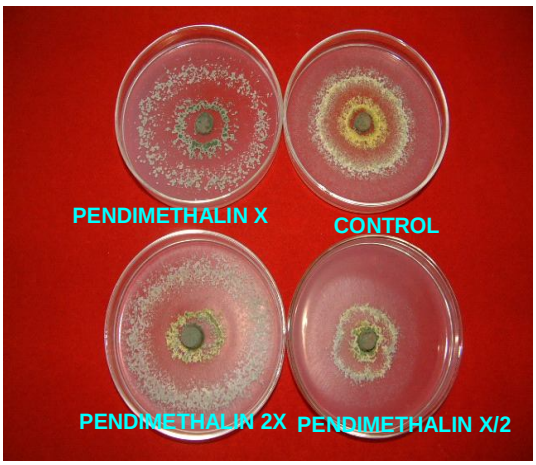
Herbicide	Radial growth (mm) of *Sclerotium rolfsii* at different intervals (hr)								
	16	24	32	40	48	56	64	72	80
1.Butachlor 0.5X	17.4	30.4	38	45.2	55.4	66.4	77.8	87.2	90
2. Butachlor X	16.6	27.4	32.8	37.8	45.8	56.4	66.4	76.8	88.4
3. Butachlor 2X	13.4	26.4	31.6	37.6	45	55	64.8	75.4	86.4
4. Alachlor 0.5X	16.8	27	34.2	43	51.6	63.8	75.8	88.2	90
5. Alachlor X	15.4	24.8	32.8	39.6	48	57.4	67.8	77.6	88
6. Alachlor 2X	15.4	27.4	33.4	39.4	48.2	56.2	66.8	77.4	88
7. Metolachlor 0.5X	8.8	11.4	16	21.4	31	42	52.6	63.4	74.4
8. Metolachlor X	11	21.6	30	33.8	43.4	54.4	64.6	76	85.2
9. Metolachlor 2X	10.8	23	31	37.4	46.8	56.8	65	79.2	87.4
10. Fluchloralin .5X	11.8	21	27.4	32.8	37.4	43.2	47.2	53.8	61.6
11. Fluchloralin X	13.6	19.6	27	32.8	37.6	43.2	48.4	55	63
12. Fluchloralin 2X	12.8	23.8	29.2	34	38.8	45.6	51.8	58.4	64.8
13. Anilophs 0.5 X	18.2	34.2	44.8	53.6	63.8	74.4	84.4	90	90
14. Anilophs X	15.4	32	41.2	50	59.6	69.6	79.6	88.2	90
15. Anilophs 2X	14.4	29.2	37.2	44.4	53.2	63.8	73.8	84.8	90
16. Metribuzin 0.5 X	15.4	34.4	43.2	51.4	63.2	74.4	85	90	90
17. Metribuzin X	15.6	28.2	35.8	41.4	52.2	64.8	76.8	87.8	90
18. Metribuzin 2X	13.6	32.8	42.2	51	61.6	72.2	81.6	90	90
19. Pendimethalin. 0.5X	12.8	26.8	34.4	41.8	46.2	54	64.8	72.8	80.8
20. Pendimethalin X	11	21.2	29.2	36	40.8	48.2	55.2	63.6	71.4
21. Pendimethalin 2X	14.6	24.8	32.8	40	45.4	52.2	59	66.6	73.6
22. Oxadiargyl 0.5X	10.8	19	24.6	32.2	38	49	58.4	67.4	75.2
23. Oxadiargyl X	16	24.6	32	40.6	46	59	69.4	76.2	83
24. Oxadiargyl 2X	15.8	26.4	33.8	40.8	44.8	54.8	64.6	72	79
25. Oxyflurfen 0.5X	15	24.2	34	43.4	49.4	61.2	72.6	81.8	87.6
26. Oxyflurfen X	16.6	30.4	38	44.8	51.6	62.2	72.4	78.2	84
27. Oxyflurfen 2X	13.6	19.4	25	30.4	32.8	40	46	52	58.4
28. Pretilachlor 0.5X	18.2	33.8	42.4	51	60.6	70.6	80.8	88.6	90
29. Pretilachlor X	17	30	35.8	44.2	52	64.2	75.8	84.8	90
30. Pretilachlor 2X	18	31.4	40.8	49.6	56.6	67.6	78	87.4	90
31. Control	17.2	23.8	32.4	40.6	51	62.6	72.8	81.8	90
LSD (P=0.05)=	1.39	1.8	1.78	2.22	1.92	4.51	5.72	4.89	3.23
Cv (%)=	7.61	5.5	4.24	4.37	3.17	4.51	6.74	5.11	3.12

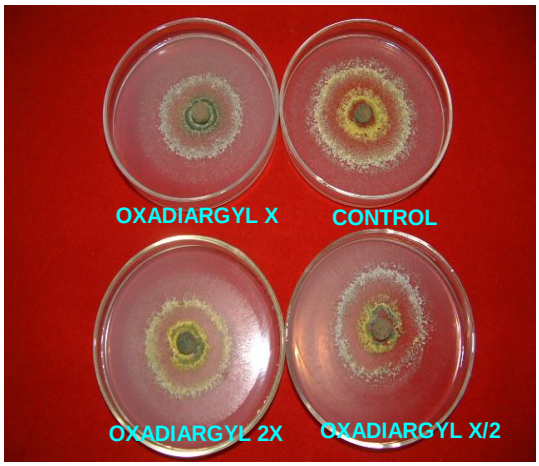
Table-3. Effect of different herbicides on radial growth of *Trichoderma* sp.

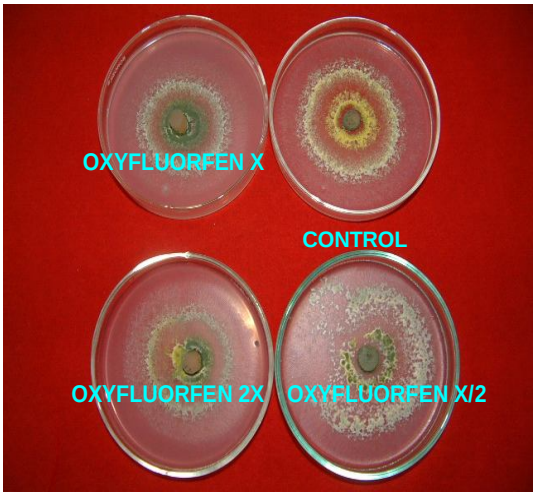
Herbicide	Radial growth (mm) of *Trichoderma* sp. at different intervals (hr)							
	16	24	32	40	48	56	64	72
1. Butachlor 0.5X	11.2	21.4	34.2	50	64.2	78.6	83.6	90
2. Butachlor X	10.4	17.6	25	38.8	51	59.6	76.6	87.8
3. Butachlor 2X	8.8	17.2	24	41.8	55.4	66.6	78.6	90
4. Alachlor 0.5X	9.2	20.4	33.2	50.2	67.2	78	84.2	90
5. Alachlor X	11.4	20.2	33.4	49	54	69.6	80.6	90
6. Alachlor 2X	11.8	20.6	34.2	49.2	64	70.4	80.4	90
7. Metolachlor 0.5X	11.4	20.8	34.6	49.4	67.6	83.4	89.2	90
8. Metolachlor X	11	21	35.2	45.4	56.6	69.6	83.2	90
9. Metolachlor 2X	8.8	19	31.2	45.2	57.8	71.4	82.6	90
10. Fluchloralin 0.5X	8.8	18.2	27.6	44	61.2	67.8	75.8	86.4
11. Fluchloralin X	8.6	17.8	28	45.4	62	77.4	84	90
12. Fluchloralin 2X	8.4	18.6	34	46.2	58.2	68	79.2	90
13. Anilophs 0.5X	12.4	21.4	34.4	48	56.4	69.8	81.4	90
14. Anilophos X	9.8	16	24.8	33.4	42	55	66.4	77.6
15. Anilophos 2X	8.6	16.6	31.8	44.8	57.2	69.2	79.8	90
16. Metribuzin 0.5 X	12.6	22	39	49.2	59.2	72.2	81.6	90
17. Metribuzin X	8.8	17.2	33	47.6	59.8	68	79.4	90
18. Metribuzin 2X	11	21.8	38	52.4	59.4	66.2	77.4	90
19. Pendimethal.0.5X	8.4	14.6	19.6	30	37.6	42.8	57.4	71.4
20. Pendimethalin X	12.4	22.2	48.2	60.2	76.6	82.6	90	90
21. Pendimethalin 2X	16.4	28.2	45.6	61	76.8	82.2	90	90
22. Oxadiargyl 0.5X	9.4	19.6	27.4	42.4	56.4	61.6	75	86.4
23. Oxadiargyl X	8.6	18.4	24.6	36	46.8	51.2	65	76
24. Oxadiargyl 2X	9.6	21	26	37.8	47.6	52.2	64.6	77.4
25. Oxyfluorfen 0.5X	10.8	20.8	34.4	46	60.2	66.6	77.2	90
26. Oxyfluorfen X	8.6	17.2	24.4	34.4	45.8	53.6	73.6	90
27. Oxyfluorfen 2X	8.8	19.6	25.8	36.6	46.4	51.2	65	76.8
28. Pretilachlor 0.5X	10.4	20.4	31.2	48.4	64.8	75.8	83.2	90
29. Pretilachlor X	8.4	17.6	32.6	43.2	53.2	59.2	72.2	84.6
30. Pretilachlor 2X	8.4	19.2	28	39	49	56.6	72.4	84
31. Control	11.6	20.8	33.8	46.8	59.6	68.4	79.4	90
LSD (P=0.05)=	1.05	1.7	1.77	4.07	3.87	3.21	3.65	3.87
Cv (%)=	8.33	6.95	4.5	7.29	5.42	3.87	3.78	3.57

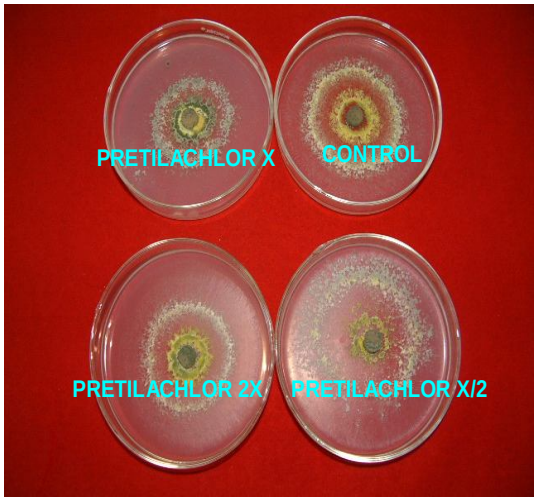
Fig.1. Effect of different herbicides on radial growth of *Trichoderma* sp.











The strong inhibitory effect of alachlor on radial growth of *S. rolfsii* was reported by Siddaramaiah *et al.*,[17] and Ekpo and Mashingaidze[4]. This is in contrast to our results where alachlor had no marked inhibition in radial growth of *S. rolfsii*. This may be due to the differences in the strain/isolate of *S. rolfsii*. pendimethalin on *S. rolfsii*, respectively. Ekpo and Mashingaidze[4] reported the inhibitory effect of metribuzin upon *S. rolfsii* at 1400 ppm. In case of our study metribuzin has interestingly stimulated the radial growth of *S. rolfsii* but the concentrations selected were 0.18, 0.37, and 0.74 ppm. It is possible that at lower concentrations metribuzin might be stimulatory to the growth of *S. rolfsii*. Kokwal *et al.*,[9] also concluded that toxicity of herbicides depends upon concentration and also varies with fungi to fungi.

Data presented in Table (3) reveals that pendimethalin at X and 2X concentrations

produced maximum radial growth of *Trichoderma* sp. (Fig.1) at all the intervals in general and were at par with metolachlor at 0.5X concentration. Herbicides like butachlor, alachlor, metolachlor (except 0.5 X) and metribuzin on an average, resulted in similar radial growth like control. Pendimethalin at 0.5X concentration reduced the radial growth of *Trichoderma* sp. Anilophos at 0.5X and 2X concentration produced at par radial growth like control. Whereas, at X concentration it reduced the radial growth of *Trichoderma* sp. Oxadiargyl at all the concentrations significantly reduced the radial growth and higher concentration of this herbicide was found more inhibitory than the lower concentration. The inhibitory effect of pendimethalin at 0.5X concentration and inducible effect at X and 2X concentrations on the radial growth of bioagent may be due to the utilization of herbicide as a nutrient source by the fungus at higher concentration.

Jayaraj and Radhakrishnan[7] reported slight inhibition in radial growth of *T. harzianum* by butachlor even at higher concentrations. In case of our studies butachlor was not having any inhibitory effect on the radial growth of *Trichoderma* sp.

Inhibitory effect of higher concentrations of alachlor on *T. harzianum* was earlier reported by Abdel-Mallek *et al.*,[1] and Jayaraj and Radhakrishnan[7]. However in our studies there was no adverse effect of this herbicide on bioagent. Baggy and Hemida[3] have reported the utilization of metolachlor by *T. harzianum*. In present study similar results were obtained on metolachlor. Fluchloralin has not been found inhibitory to radial growth of *Trichoderma* sp.

Metribuzin at all the concentrations was not found to have any inhibitory effect on *Trichoderma* sp. Tillyakhodzhaeva[18] has also reported the same effect of metribuzin on *Trichoderma* sp. Strong inhibition in the radial growth of *T. harzianum* and *T. viride* was reported by Jayaraj and Radhakrishnan[7] and Jaworska and Dluzniewska[5]. However, in our studies pendimethalin was found to be inhibitory to the bioagent only at lower concentration but stimulatory at higher concentration. This may be due to efficacy of this *Trichoderma* sp. (BMIR isolate) to utilize pendimethalin as a food source.

Jayaraj and Radhakrishnan[7] reported about inhibitory effect of oxyfluorfen on *Trichoderma* sp. In present studies higher doses of oxyfluorfen have also reduced the radial growth of bioagent. Whereas at lower concentration the herbicide produced at par growth with control.

Radial growth of *S. rolfsii* was stimulated by herbicides anilophos, metribuzin, and pretilachlor. Fluchloralin drastically reduced the radial growth of pathogen. Radial growth of *Trichoderma* sp. (BMIR isolate) was stimulated by pendimethalin at X and 2X and metolachlor at 0.5X concentrations under *in vitro* conditions. Herbicide oxadiargyl inhibited the radial growth of bioagent and its higher concentrations were more inhibitory than the lower one.

In last it is concluded that appli-cation of herbicides has variable effects on biocontrol activity of *Trichoderma* spp. It is not necessary that a herbicide affecting one mechanism of

antagonism will necessarily affect rest of the mechanisms exhibited by the bioagents. Herbicides like alachlor that in general showed positive effects on bioagent and negative effects on pathogen may be a good recommendation (if provided by further necessary confirmations) for an integrated crop management system.

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