





more sensitive to Rizolex T 50 % than *T. harzianum* (Table 3).

#### Radial growth of Pathogenic fungi at different concentrations of Rizolex T 50 %:

*Fusarium proliferatum*, *Fusarium oxysporum* and *Acremonium strictum* exhibited a different ability to grow on different concentrations of Rizolex T 50 % in growth medium at different incubation periods. The growth of all tested pathogenic fungi greatly decreased with increasing fungicide concentration in the growth medium comparing with the control. After eight days, *F. oxysporum* and *F. proliferatum* were able to grow at 450, 550 ppm respectively of Rizolex T 50 % with percentage inhibition of 84.4% and 78.8% and they cannot able to grow above these concentrations. In contrast, *Ac. strictum* was the most sensitive one to Rizolex T 50 %, as it was not able to grow above 250 ppm of Rizolex T 50 % with percentage inhibition 80% at this concentration after eight days. 50% inhibition of *F. proliferatum* was approximately obtained at 250ppm of Rizolex T 50 %, while it was obtained at 150 ppm for *F. oxysporum* and less than 50 ppm for *Ac. strictum*, (Table 4).

#### Antagonistic activity of *T. harzianum* and *T. koningii* toward the pathogenic fungi in the presence of different concentrations of Rizolex T 50%:

The growth of pathogenic fungi in absence of *T. harzianum* in the control medium without Rizolex T50% reached to approximately 75mm colony diameter for *F. oxysporum* and *F. proliferatum* and 50mm for *Ac. strictum* whereas, in the presence of *T. harzianum* it sharply decreased to 17, 20 and 11 mm respectively. Therefore its antagonistic effect was very high and reached to 77.3, 77.5 and 78% reduction in the growth of the pathogenic fungi respectively (Table 5).

The presence of Rizolex T 50% in the growth medium highly decreased the growth of *T. harzianum* and pathogenic fungi. The antagonistic effect of *T. harzianum* against pathogenic fungi slightly increased in the presence of the fungicide Rizolex T50%. At 200 ppm Rizolex T in combination with *T. harzianum* reduced the growth of *F. oxysporum* and *F. proliferatum* and *Ac. strictum* to approximately 86.6, 85 and 100% respectively. While, with *T. koningii* in combination with Rizolex T at 300 ppm, the growth of these pathogenic fungi reduced to approximately 80, 81.1 and 100% respectively. Utilization of low dose of fungicide in combination with *Trichoderma spp.* increased the inhibition percentage of pathogenic fungi and may be beneficial in controlling these fungi.

## 4. Discussion

In recent years, research on biological control has gained momentum for controlling serious soil born plant pathogens like *Fusarium*, *Rizoctonia*, *Macrophomina*, *Sclerotium*, *Pythium* and *Phytophthora spp.*, employing *Trichoderma* and *Gliocladium* species and varied success has been achieved. Several *Trichoderma spp.* could be effectively used in biocontrol of soil borne plant pathogens, *Trichoderma spp.* are active as hyperparasites [13].

In this work, the antagonistic activity of isolated *Trichoderma harzianum* was high against pathogenic fungi *F. oxysporum*, *F. proliferatum* and *Ac. strictum* and reached to 77.3, 77.5 and 78% reduction respectively. While, *T. koningii* reduced the growth of these fungi to approximately 69, 73 and 77% respectively. Several reports have indicated that biocontrol efficiency of *Trichoderma spp.* against *Fusarium* wilts may differ in different regions of the world i.e., a highly antagonistic species against a particular pathogen in a given region may react poorly against the same pathogen in another region[14],[15]. In a study carried out by [16], the efficacy of *T. koningii* for the suppression of *F. oxysporum* reached to about 91% in vitro while, in this study, *T. koningii* was able to reduce it to 69.3%.

*T. harzianum* was able to grow in the presence of Rizolex T50% up to 400 ppm with percentage inhibition of 83.3% while, *T. koningii* cannot grow at this concentration but grown up to 350 ppm with percentage inhibition of 87.7%. Similar result was also observed by [17], they observed that mycelial growth of *Trichoderma spp.* was most sensitive to Benlate, Ridomil gold, Tecto-60 and Topsin-M at both the concentrations of 100 and 200ppm. Similar results concerning the inhibitor effect of Rizolex-T 50% and Topsin-M at different concentrations against soil borne fungi, *R. solani* and *F. oxysporum* were also observed by [18].

In our results the combination of *Trichoderma spp.* with low concentration of Rizolex T lead to increase inhibition of pathogenic fungi (*F. oxysporum*, *F. proliferatum* and *A. strictum*) in compare with *Trichoderma* or low concentration of Rizolex T separately. This result agrees with [19], they reported that the efficacy of the *Trichoderma* treatments has been both more variable and less effective than chemical fungicide treatments. Combining bio-control agents with selective fungicides that are not inhibitory to the antagonist will help it to become established in the soil and achieve better disease control and also with[20], who demonstrated an improved disease control of gladiolus corm rot and wilt caused by *F. oxysporum* by combining an isolate of *T. virens* and the fungicide carboxin. Analogous results were obtained by [21], with mutants of benomyl-tolerant strains of *T. pseudokoningii*, which were superior to the wild type in bio-control potential on *S. rolfsii*.

Apparently, biocontrol with *Trichoderma* has to be implemented with other disease control measures to reach a satisfactory level of disease control. A correlation between fungicide resistance and antagonistic activity is suggested by [22], affirming that the up regulated expression of ABC transporter genes of *T. atroviride* during the three-way interaction with various plants and fungal pathogens, possibly supports both antagonistic activity and root colonization.

*In conclusion:* Isolation and selection of fungicides tolerant strains of *Trichoderma* species may help in better controlling of pathogenic fungi. Application of low doses of rizolex T50% in combination with biologically control *Trichoderma* species highly increased the inhibition ratio of pathogenic fungi and therefore, better control of plant diseases.

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**Table 1:** Radial growth (mm) of *Trichoderma* isolates grown on PDA medium amended with different concentrations of Rizolex T 50 % fungicides after six days. Data are the mean value of 3 replicates ± standard error of mean.

Rizolex T 50 % (PPM)	T1	T2	T3	T4	T5	T6
0	87±0.10	90±0.00	90±0.00	90±0.00	90±0.00	90±0.00
50	20±0.08	17±0.08	23±0.04	28±0.10	41±0.04	60±0.04
100	13±0.08	11±0.04	16±0.10	22±0.11	32±0.08	44±0.08
150	0.0	0.0	11±0.08	14±0.10	28±0.08	35±0.08
200	0.0	0.0	0.0	9±0.04	23±0.10	32±0.12
250	0.0	0.0	0.0	0.0	20±0.04	29±0.04
300	0.00	0.0	0.0	0.0	18±0.04	23±0.04
350	0.00	0.0	0.0	0.0	11±0.08	19±0.08
400	0.00	0.0	0.0	0.0	0.0	15±0.04

**Table 2:** Antagonistic effect of *T. harzianum* and *T. koningii* against pathogenic fungi, Data are expressed as colony diameter (mm) after 6 days of incubation, mean of 3 replicates ± standard error of mean.

	<i>Trichoderma</i> against <i>F. oxysporum</i>			<i>Trichoderma</i> against <i>F. proliferatum</i>			<i>Trichoderma</i> against <i>Ac. strictum</i>		
	<i>Trichoderma</i>	<i>F. oxysporum</i>	% reduction	<i>Trichoderma</i>	<i>F. proliferatum</i>	% reduction	<i>Trichoderma</i>	<i>Ac. strictum</i>	% reduction
Control	--	75±0.10	--	--	75±0.10	--	--	50±0.08	--
<i>T. harzianum</i>	39±0.12	17±0.12	77.3	38±0.06	20±0.12	73.3	40±0.06	11±0.06	78
<i>T. koningii</i>	30±0.10	23±0.07	69.3	31±0.12	20±0.12	73.3	35±0.03	17±0.10	77.3

**Table 3:** Effect of different concentrations of Rizolex T 50 % on radial growth of *T. harzianum* and *T. koningii*. Data are the mean value of 3 replicates ± standard error of means.

Fungicide concentration	<i>T. harzianum</i>				<i>T. koningii</i>			
	2 day	4 day	6 day	Inhibition%	2 day	4 day	6 day	Inhibition%
0 ppm	58±0.07	83±0.07	90±0.00	00	55±0.08	80±0.04	90±0.00	00
50 ppm	25±0.06	43±0.03	60±0.04	33.3	14±0.08	28±0.08	41±0.04	54.4
100 ppm	18±0.12	30±0.06	44±0.08	51.1	12±0.08	21±0.08	32±0.08	64.4
150 ppm	13±0.10	24±0.07	35±0.08	61.1	11±0.00	19±0.08	28±0.08	68.8
200 ppm	12±0.10	23±0.10	32±0.12	64.4	8±0.00	16±0.08	23±0.10	74.4
250 ppm	11±0.10	20±0.09	29±0.04	67.7	8±0.00	12±0.00	20±0.04	77.7
300 ppm	10±0.05	16±0.09	23±0.04	74.4	8±0.00	12±0.08	18±0.04	80
350 ppm	8±0.03	13±0.09	19±0.08	78.8	8±0.00	9±0.03	11±0.08	87.7
400 ppm	8±0.00	10±0.09	15±0.04	83.3	8±0.00	8±0.00	8±0.00	-

**Table 4:** Effect of different concentrations of Rizolex T 50% on radial growth of *Fusarium proliferatum*, *Fusarium oxysporum* and *Acremonium strictum*. Data are the mean value of 3 replicates ± standard error of means.

Fungicide conc.	<i>F. proliferatum</i>				<i>F. oxysporum</i>				<i>Ac. strictum</i>			
	4 day	6 day	8 day	I%*	4 day	6 day	8 day	I%	4 day	6 day	8 day	I%
0ppm	49±0.0	68±0.08	90±0.0	00	57±0.0	79±0.09	90±0.0	00	31±0.07	49±0.0	63±0.0	00
50ppm	34±0.0	49±0.08	65±0.0	27.7	23±0.0	39±0.03	55±0.0	38.8	15±0.07	23±0.0	31±0.0	65.5
100ppm	28±0.0	42±0.04	57±0.0	36.6	20±0.0	32±0.06	48±0.0	46.6	13±0.03	17±0.0	24±0.0	73.3
150ppm	24±0.0	37±0.04	50±0.0	44.4	17±0.1	27±0.07	40±0.0	55.5	11±0.07	15±0.0	21±0.0	76.6
200ppm	21±0.1	33±0.08	47±0.0	47.7	14±0.0	25±0.07	36±0.0	60.0	9±0.03	12±0.0	18±0.0	80
250ppm	19±0.0	30±0.08	44±0.0	51.1	12±0.0	20±0.03	32±0.0	64.4	8±0.03	9±0.07	11±0.0	87.7
300ppm	16±0.1	27±0.10	40±0.1	55.5	11±0.0	19±0.03	29±0.0	67.7	0.00	0.00	0.00	
350ppm	14±0.0	25±0.04	36±0.0	60	11±0.0	16±0.03	26±0.0	71.1				
400ppm	12±0.1	21±0.12	34±0.1	62.2	10±0.0	14±0.07	20±0.0	77.7				
450ppm	11±0.0	18±0.04	29±0.0	67.7	10±0.0	11±0.03	14±0.0	84.4				
500ppm	10±0.0	14±0.08	23±0.0	74.4	0.00	0.00	0.00					
550ppm	8±0.04	12±0.04	19±0.0	78.8								

\* I% expressed inhibition percentage

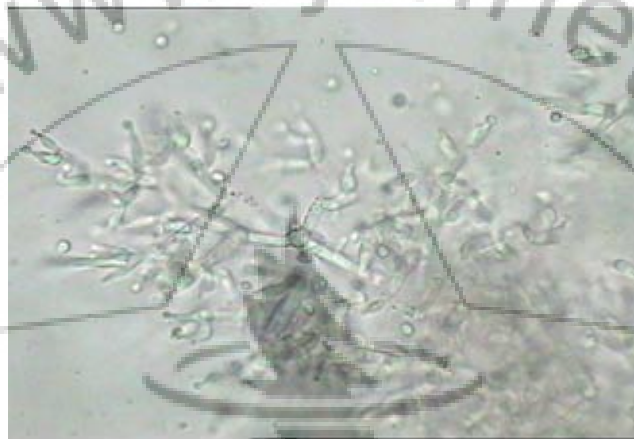
**Table 5:** Antagonistic effect of *Trichoderma harzianum* and *T. koningii* against pathogenic fungi in the absence and presence of fungicide. Data are expressed as colony diameter (mm) after 6 days of incubation, mean of 3 replicates ± standard error of mean.

<i>Trichoderma</i> strains	Rizolex (ppm)	<i>Trichoderma F. oxysporum</i>			<i>Trichoderma F. proliferatum</i>			<i>Trichoderma Ac. strictum</i>		
		Colony diameter (mm)	Colony diameter (mm)	% reduction	Colony diameter (mm)	Colony diameter (mm)	% reduction	Colony diameter (mm)	Colony diameter (mm)	% reduction
<i>T. harzianum</i>	Cont.	-	75±0.10	-	-	75±0.10	-	-	50±0.08	-
	0	39±0.12	17±0.12	77.3	38±0.06	20±0.12	73.3	40±0.06	11±0.06	78
	100	29±0.12	15±0.15	80	32±0.06	20±0.12	75	30±0.06	10±0.07	80
	200	24±0.32	10±0.15	86.6	21±0.23	13±0.10	85	25±0.12	-	100
	300	20±0.12	11±0.07	85.3	20±0.18	12±0.12	86.3	22±0.07	-	-
	400	16±0.12	11±0.07	85.3	17±0.18	12±0.10	86.3	-	-	-
<i>T. koningii</i>	Cont.	-	75±0.10	-	-	75±0.10	-	-	50±0.08	-
	0	30±0.10	23±0.07	69.3	31±0.12	20±0.12	73.3	35±0.03	17±0.10	77.3
	100	25±0.12	24±0.12	68	24±0.06	18±0.06	76	25±0.03	11±0.23	78
	200	20±0.09	19±0.12	74.3	20±0.15	14±0.12	81.1	21±0.12	10±0.25	80
	300	14±0.06	15±0.06	80	15±0.23	14±0.15	81.1	15±0.12	-	100



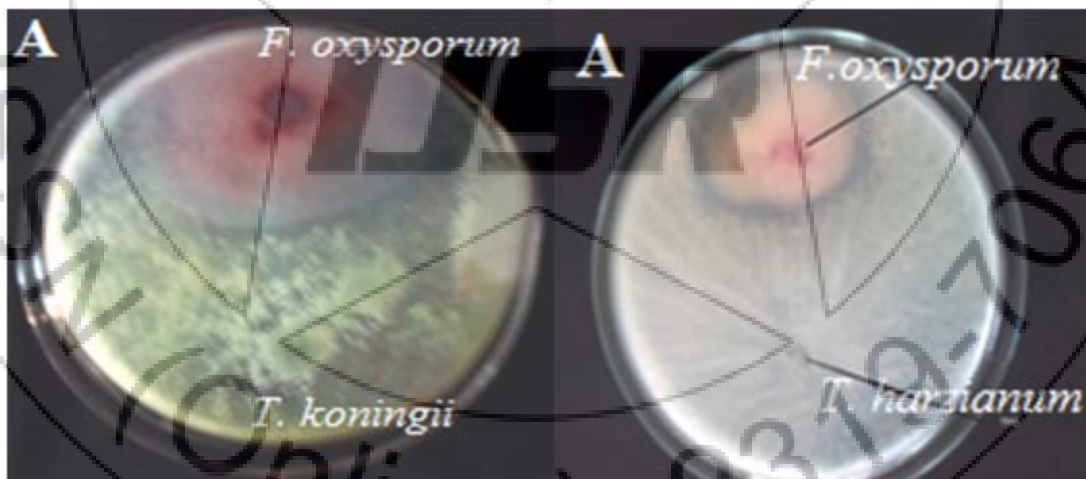


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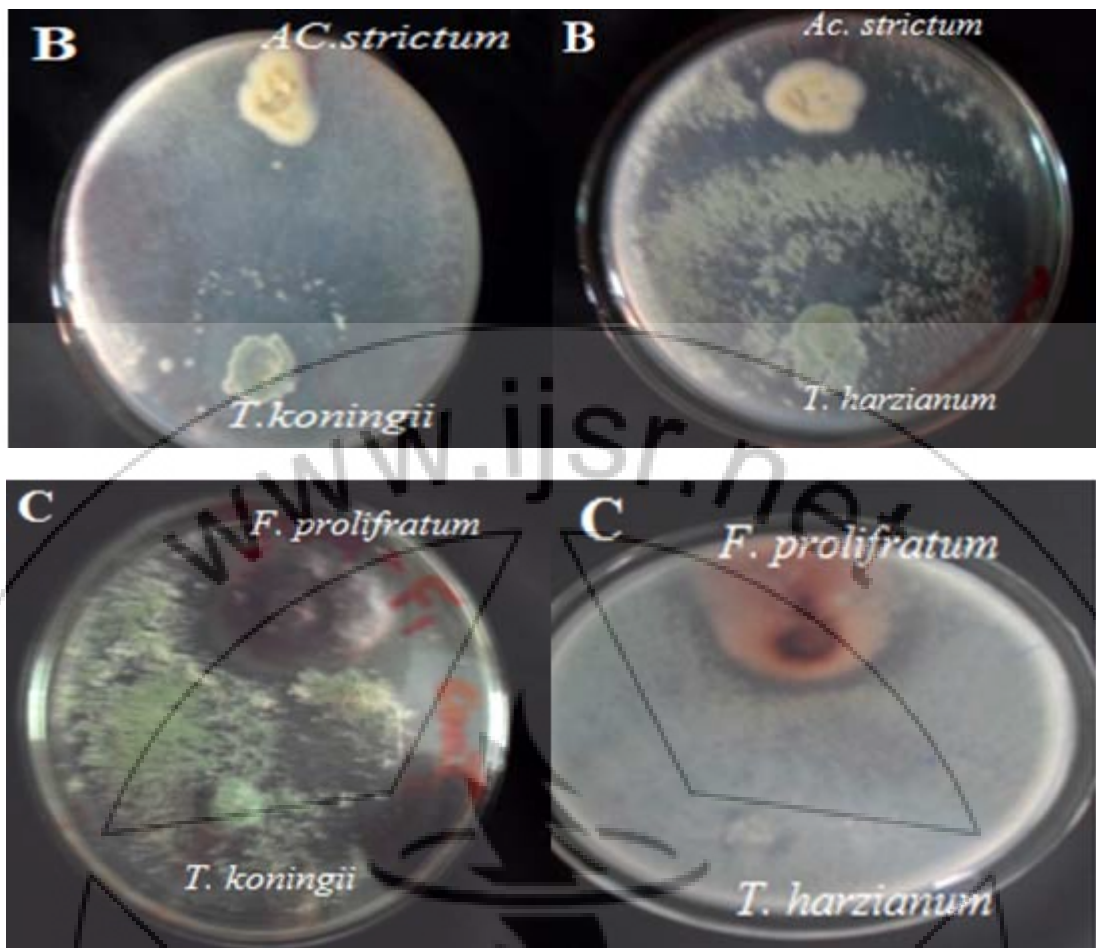


B

Figure 1: Photomicrograph shows the phialides and conidia of (A) *Trichoderma koningii* and (B) *Trichoderma harzianum* under microscope.



A *F. oxysporum* A *F. oxysporum*  
*T. koningii* *T. harzianum*



**Figure 3:** Photograph shows antagonistic activity of *T. koningii* and *T. harzianum* against pathogenic fungi (A) *F. oxysporum*, (B) *Ac. strictum* and (C) *F. proliferatum*.

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