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The Potential Effects of Fungicides on Association of *Rhizophagus irregularis* with Maize and Wheat

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HIGHLIGHTS

- The fungicides in the laboratory experiment impaired root colonization of *R. irregularis*.
- In the greenhouse experiment, fungicides had no negative effect on root colonization of *R. irregularis*.
- The benomyl in maize and tilt in wheat and rovral TS in both plants could be recommended with the combined application of *R. irregularis*.
- Depending on fungicide type and host plant, the effect of fungicide on *R. irregularis* varies.

Abstract: The effect of different fungicides on mycorrhizal fungi should be investigated in different plants and environmental conditions. Thus, the purpose of this study was to appraise the effect of simultaneous fungicides application (including benomyl, rovral TS, mancozeb, and tilt) on the efficiency of *Rhizophagus irregularis* in cultivations of maize and wheat. This study was conducted in two separate experiments in the laboratory and greenhouse. The results of the laboratory stage showed that the use of all four fungicides significantly reduced the spore number compared to the conditions of non-use of the fungicide, although only rovral TS and mancozeb led to a significant reduction in root colonization percentage of *R. irregularis*. In the greenhouse, the benomyl significantly increased root dry weight in maize although tilt significantly reduced root colonization of maize with *R. irregularis*. The tilt and rovral TS had a positive effect and benomyl had a negative effect on wheat growth traits, but the root colonization of wheat with *R. irregularis* was not affected by fungicides. Generally, benomyl (2 g L^{-1}) in maize and tilt (2 mL L^{-1}) in wheat and rovral TS in both plants could be recommended with the combined application of *R. irregularis* inoculants. Therefore, depending on the type of fungicide and the host plant, the effect of the fungicide on colonization and association of mycorrhiza varies.

Keywords: Fungicides; Mycorrhizal symbiosis; Root colonization; Sporulation.

INTRODUCTION

Pathogenic fungi cause many diseases for the plants and therefore reduce their growth and yield. Various methods such as chemical fungicides and biocontrol agents such as endomycorrhizal fungi are used to control these pathogens. Fungicides are being utilized to control plant pathogenic fungi with targeting different biological processes such as disruption in cell function, impose blockades in ergosterol biosynthesis, protein biosynthesis (tubulin) or essential enzymes (cytochrome c reductase) [1, 2]. Coating seeds with fungicides is one of the common solutions in order to prevent primary fungal attacks. This approach is being used successfully in controlling seed pathogens and seedling wilting pathogens [3].

Among fungicides, benomyl, rovral TS, mancozeb, and propiconazole have been widely used against plant pathogenic fungi. Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate, $C_{14}H_{18}N_4O_3$], is a systemic wide-spectrum agricultural fungicide used to control certain fungal diseases of stone fruit, powdery mildews and soil-borne pathogens [4]. Rovral TS is the combination of non-systemic iprodione (3-(3,5-dichlorophenyl)-2,4-dioxo-N-propan-2-ylimidazolidine-1-carboxamide) and systemic carbendazim (methyl N-(1H-benzimidazol-2-yl) carbamate) fungicides, which made it a broad-spectrum fungicide. Carbendazim has an impact on β-tubulin hence inhibits mitosis, iprodione but affects kinase proteins that are involved in phosphorylation [5, 6]. Mancozeb is a protectant contact fungicide from dithiocarbamate group [a chemical mixture of “zinc; manganese(2^+); N-[2-(sulfidocarbothioylamino)ethyl]carbamodithioate, $C_8H_{12}MnN_4S_8Zn$]. This fungicide is broad-spectrum and relatively stable that causes general disorder in cell metabolism. Mancozeb is being used to control smut and other plant pathogenic fungi [7]. Propiconazole with the trade name of “Tilt” is a systemic fungicide, which belongs to the triazoles group. This fungicide applied against a broad range of pathogenic fungi like rice sheath blight, wheat rust, *Fusarium* head blight of wheat [8].

Due to the dependency of industrial agriculture on pesticides, the excessive use of these chemical compounds has led to negative impacts on soil ecology and subsequently on beneficial or plant probiotic soil microflora [9]. The impact of pesticides on soil microorganisms have been investigated under field [10], greenhouse [11] and growth chamber [12]. Even by a single administration of pesticides, the residue will remain in the ground for a long time hence has destructive effects on the soil microorganisms [13]. Pesticides could be absorbed on soil particles and microorganisms, thereby deleterious impacts on non-target sites would lessen. Soil types profoundly affect the availability of pesticides, each soil type could differently affect each pesticide [9].

The policies for sustainable agriculture have encouraged experts to apply soil microorganisms in order to protect plants against pathogens as well as supplying nutrients more than before [14, 15, 16, 17, 18, 19]. Mycorrhizal fungi are among the most important microorganisms in agricultural soil [20]. Arbuscular mycorrhizal (AM) symbiosis occurs in at least 80% of the vascular plant families. AM fungi increase nutrients uptake such as phosphorus and nitrogen by plants through increasing the absorbing surface area and mobilizing sparsely available nutrients. Plant hosts also supply AM fungi with a carbon source that is essential for fungal growth. Studies have shown that lipids are transferred from the plant hosts to AM fungus as a major carbon source [21]. Mycorrhizal symbiosis has positive effects on the quantitative and qualitative characteristics of the plant hosts in conditions of biotic and abiotic stresses [22, 23, 24, 25]. Other benefits like increment in photosynthesis, improving soil physical condition through hyphae spreading in the soil profile, preventing toxic ions from being absorbed and more importantly controlling root pathogenic agents are attributed to mycorrhizal symbiosis [26]. Plants with mycorrhizal symbiosis usually endure pathogenic fungi better than non-mycorrhizal plants [27]. It is reported that colonization of soybean with *Glomus mosseae* induced resistance in soybean encountering plant pathogenic fungi such as *Fusarium solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani*, while non-mycorrhizal soybean showed stunted growth [28]. Tomato colonized with mycorrhizal fungi was less susceptible to *Phytophthora* [29]. Mycorrhizal fungi could induce resistance to pathogens directly through making a physical barrier on roots, and indirectly with improving nutritional condition thereby accelerating the plant growth. In addition, the physical presence of mycorrhizal fungi could impede the infection of other fungi. Another possibility is that plant or mycorrhizal fungi generate anti-pathogenic compounds like antibiotics that inhibit pathogenic infection [30, 31].

Fungicides and mycorrhizal fungi are sometimes being applied together in which fungicides could have negative impacts on mycorrhizal fungi. Scientists reported that carbendazim and mancozeb restricted mycorrhizal infection on groundnut and maize [32]. It has been also disclosed that due to deleterious effects, benomyl must be avoided in order to preserve arbuscular mycorrhizal fungi [33]. Propiconazole, one of the

most frequently used systemic fungicides was reported to have side-effects on mycorrhizal fungi, whereas it is an ergosterol inhibitor thereby should not have the major impact on arbuscular mycorrhizal fungi, which contain only small amounts of ergosterol. However, the detrimental effects of propiconazole have been reported on root colonization, plant growth and spore production [34]. Controversially, some fungicides had no deleterious effects on AM (arbuscular mycorrhizal) fungi and even in some cases increased colonization and nutrient uptake, especially at lower application dosages. In general, the effects of pesticides on soil beneficial microorganisms will vary depending on the chemical dosage, soil properties and various environmental factors [9]. Hence, the effect of different fungicides on the mycorrhizal fungi should be investigated in different plant species and environmental conditions. Therefore, this experiment was conducted to answer these ambiguities more accurately. The specific objectives of this study were to: (i) appraise the effect of some systemic and contact fungicides on the colonization and sporulation of arbuscular mycorrhizal fungi in *in vitro*, (ii) investigate the symbiotic association of *Rhizophagus irregularis* with maize and wheat plants, and (iii) survey mycorrhizal efficiency for improving morphological traits of the host plants.

MATERIAL AND METHODS

Laboratory experiment

The capability of growth and proliferation of *R. irregularis* in the growth media containing different concentrations of fungicides were evaluated. *R. irregularis* was taken from the Soil and Water Research Institute, Karaj, Iran. For the proliferation of *R. irregularis*, sterile glass plates containing 100 mL MS media with 0.3% phytagel were inoculated with colonized carrot roots. Afterwards, plates were sealed with Parafilm and moved to the incubator (28˚C) for 12 weeks.

Fungicides including benomyl, rovral TS, mancozeb, and tilt were purchased from Agricultural Eksir Company. Different concentrations of these fungicides (0.5, 1, 2 and 3 g L^{-1} of benomyl, rovral TS, and mancozeb; 0.5, 1, 2 and 3 mL L^{-1} of tilt) were mixed in MS medium. The amount of 50 mL of growth media containing each concentration of fungicides was poured in glass jars. Afterwards, equal pieces of colonized carrot roots with *R. irregularis* were cultivated in jars. Jars were incubated for 2 months at 28˚C. Then, the quantitative characteristics including spore numbers in the growth media and root colonization were assessed.

Greenhouse experiment

Low-fertile soil, which was not cultivated for several years, was collected from the Soil and Water Research Institute (SWRI), Karaj, Iran. Soil sampling was performed at a depth of 0-30 cm. In order to remove stone particles, a sieve with a mesh size of 0.5 cm diameter was used. Soil texture was determined using the hydrometer method [35]. Soil pH and EC were measured through the saturated extract method. Soil nitrogen using the Kjeldahl method, available potassium by the flame photometry [36] and available phosphorus by Olsen method [37] was measured. The wet oxidation method was also used to measure the amount of organic carbon [38]. The soil was extracted by DTPA (diethylenetriaminepentaacetic acid), and the amount of Fe, Cu, Mn, and Zn were determined by using Atomic Absorption Spectrometer [39] (Table 1).

Table 1. Soil physiochemical characteristics (0-30 cm).

Soil texture	pH	EC	N	OC	P	K	Fe	Zn	Mn	Cu
		dS m^{-1}	%		mg kg^{-1}					
Loam	7.7	1.01	0.07	0.72	7.9	233	2.16	0.46	7.88	1.02

EC= Electrical Conductivity; SP= Saturation Percentage; T.N.V.= Total Neutralizing Value; OC= Organic Carbon.

Four-Kg plastic pots were filled with a mixture of soil, peat moss, and perlite in the ratio of 4, 1, and 1, respectively. Maize (*Zea mays*, single cross 704) and wheat (*Triticum aestivum*, Chamran) seeds were shaken in each fungicide solution at the concentration of 2 g L^{-1} (benomyl, rovral TS, mancozeb) and 2 mL L^{-1} (tilt) for 30 min. Afterwards, they were transferred to the plates containing water agar and moved to the incubator for germination. In order to apply mycorrhizal fungus, 25 g of *R. irregularis* inoculant with 200 active propagules per gram was layered at 3 cm below the soil surface. In each pot, three germinated seeds of

wheat or maize were cultivated and covered with the soil layer. Soil moisture in pots was maintained at 80% of the field capacity. Based on soil analysis, 100 mg nitrogen in the form of urea was added to each pot.

Two months after starting the experiment, shoot height was measured. The aerial part of the plants was cut at the soil surface and the roots were separated from the soil and then the shoot and root fresh weights of plants were measured. The aerial part and root of the plants were dried in the oven at 70°C for 72 h and the shoot and root dry weights were determined. One gram of fresh roots after washing with water was maintained in FAA solution (Formalin, Acetic acid, and Alcohol) until the root colonization percentage was assessed.

Root colonization percentage of *Rhizophagus irregularis*

The percentage of root colonization with *R. irregularis* was assessed with the gridline intersection method [40, 41]. Root samples were removed from the preserving solution and washed thoroughly with water, then transferred to the test tubes and dyed with Trypan blue. For each sample, 100 roots were cut with a scalpel and were randomly placed on the 9-cm diameter Petri plate with gridlines. Active propagules like hyphae, spore, vesicle, and arbuscule were estimated using a stereomicroscope (Leica ZOOM 2000, USA). The level of colonization was evaluated through quantifying the colored and uncolored intersections between horizontal and vertical lines and roots [41,42].

Statistical analysis

The one-way analysis of variance was done for both laboratory and greenhouse experiments and mean comparisons were performed using Duncan's multiple range tests through the general linear model (GLM) procedure in SAS 9.1 software. Laboratory experiment for investigating the effects of fungicides on the root colonization percentage and sporulation was conducted in a complete randomized design with four concentrations of fungicides and control, within five repetitions. Simple linear regression was applied to find out how spore number varies with root colonization percentage. The linear regression graph was drawn by XLSTAT. The greenhouse experiment was also conducted in a complete randomized design with five levels of fungicide treatments in four repetitions for each plant. Graphs were drawn in Excel.

RESULTS

Laboratory experiment

According to the analysis of variance, fungicide treatments affected both root colonization and sporulation of *R. irregularis* ($p \leq 0.01$). The results showed that fungicides rovral TS and mancozeb at all concentrations led to a significant reduction in root colonization percentage compare to control but the decreasing effect of fungicides benomyl and tilt on root colonization was not significant. Also, no significant difference was observed between benomyl and tilt at the similar concentrations in terms of the effect on root colonization percentage. The use of all four fungicides significantly reduced the spore number compared to the conditions of non-use of the fungicide, although the decreasing effect of rovral TS and mancozeb was greater than that of benomyl and tilt. There were reducing trends in root colonization percentage and spore number with increasing fungicide concentrations, even though no significant differences were observed between concentrations (Table 2).

Table 2. Mean comparison of fungicide treatments on root colonization percentage and spore number in the in vitro experiment.

Treatments		Root colonization (%)	No. of spore (No. in mL liquid)
No use of fungicides		68.87 a	81.00 a
Benomyl	0.5 g L^{-1}	57.12 ab	39.00 b
	1 g L^{-1}	63.37 a	31.20 bc
	2 g L^{-1}	57.55 ab	30.40 bc
	3 g L^{-1}	60.29 ab	39.00 b
Rovral TS	0.5 g L^{-1}	26.50 cd	8.00 cd
	1 g L^{-1}	25.52 cd	7.80 cd
	2 g L^{-1}	21.23 d	7.40 d
	3 g L^{-1}	18.89d	6.20 cd
Mancozeb	0.5 g L^{-1}	37.23 bcd	4.60 d
	1 g L^{-1}	17.64 d	2.00 d
	2 g L^{-1}	14.51 d	1.80 d
	3 g L^{-1}	15.49 d	1.40 d
Tilt	0.5 mL L^{-1}	50.60 ab	15.60 bcd
	1 mL L^{-1}	51.13 ab	14.20 cd
	2 mL L^{-1}	51.01 ab	12.00 cd
	3 mL L^{-1}	46.33 abc	11.60 cd

Different letter(s) showed significantly differences between treatments (Duncan p≤0.05).

Simple linear regression of spore numbers by colonization percentage for all the fungicide treatments illustrated that 62% of the variability of spore numbers could be explained by the root colonization percentage (Figure 1). Coefficient intervals around the regression line exhibit a range of spore number variability affected by changing the root colonization percentage.

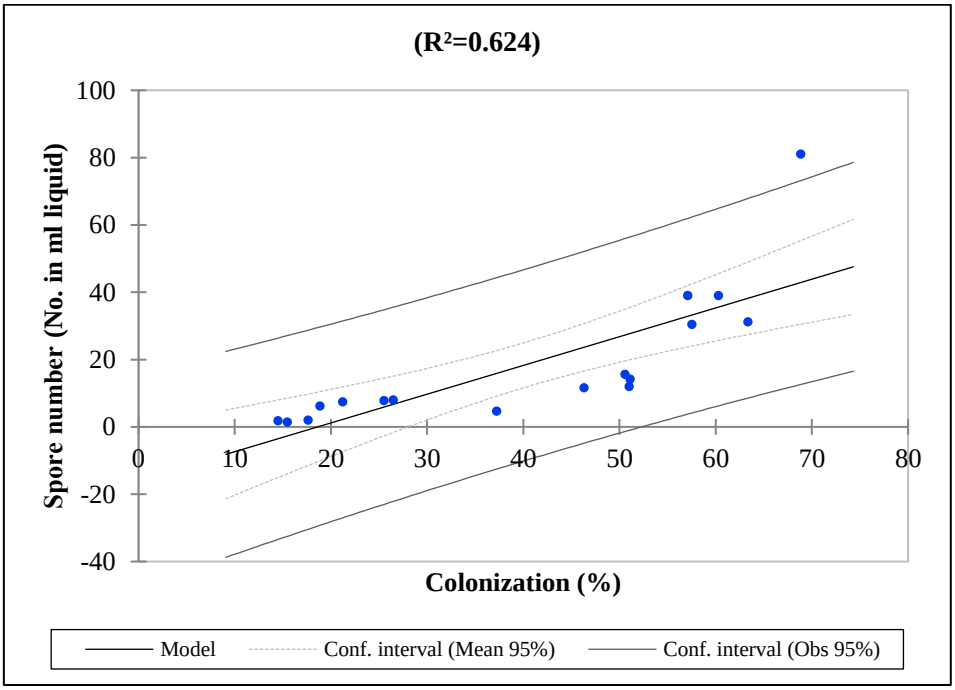

Figure 1. Linear regression of spore number by colonization percentage for all the fungicide treatments.

As there were not any significant differences between fungicide concentrations for these investigated traits (root colonization percentage and number of spores), hence we only applied the recommended rates (2 g L^{-1} of benomyl, rovral TS, and mancozeb; and 2 mL L^{-1} of tilt) for the greenhouse experiment.

Greenhouse experiment

Maize plants

Analysis of variance showed that among the investigated morphological parameters in maize, root dry weight was the only parameter that significantly affected by the fungicide treatments ($p \leq 0.05$). There were no significant effects on plant height, root fresh weight, shoot fresh and dry weights, and root colonization percentage. Mean comparison (Table 3) indicated that benomyl, significantly increased root dry weight in maize although other fungicides had not shown any significant difference with control.

Fungicide treatments did not show significant effects on the shoot fresh weight in maize plants, albeit the highest and lowest amounts were seen in rovral TS and tilt treatments, respectively. Similar results were observed for the shoot dry weight, in that case the highest and lowest amounts were observed by benomyl and mancozeb application, respectively (Table 3).

Table 3. Mean comparison of fungicide treatments on maize morphological traits and root colonization percentage.

Treatments	RF	RD	SF	SD	SL	Root colonization
	(g pot^{-1})				(cm)	(%)
Control	20.95 a	2.42 b	71.73 a	21.44 a	91.29 a	40.96 ab
Benomyl	29.52 a	3.24 a	80.15 a	24.08 a	91.75 a	44.28 a
Rovral TS	22.06 a	2.36 b	80.96 a	22.48 a	88.25 ab	34.57 ab
Mancozeb	24.44 a	2.69 ab	75.28 a	20.49 a	90.20 ab	36.70 ab
Tilt	24.99 a	2.65 ab	69.14 a	21.53 a	86.41 b	30.16 b

Different letter(s) showed significantly differences between treatments (Duncan $p \leq 0.05$).
RF: Root Fresh weight; RD: Root Dry weight; SF: Shoot fresh weight; SD: Shoot Dry weight; SL: Shoot Length.

Fungicide treatments had not exerted any significant effects on plant height as well, even though benomyl and tilt treatments showed the highest and lowest plant heights (Table 3). Root colonization percentage in maize plants was not significantly affected by the fungicide treatments. Although the highest and lowest root colonization percentages were observed in benomyl and tilt treatments, respectively (Figure 2a). There was no significant difference between benomyl treatment and control, even plants treated with benomyl showed higher root colonization percentage.

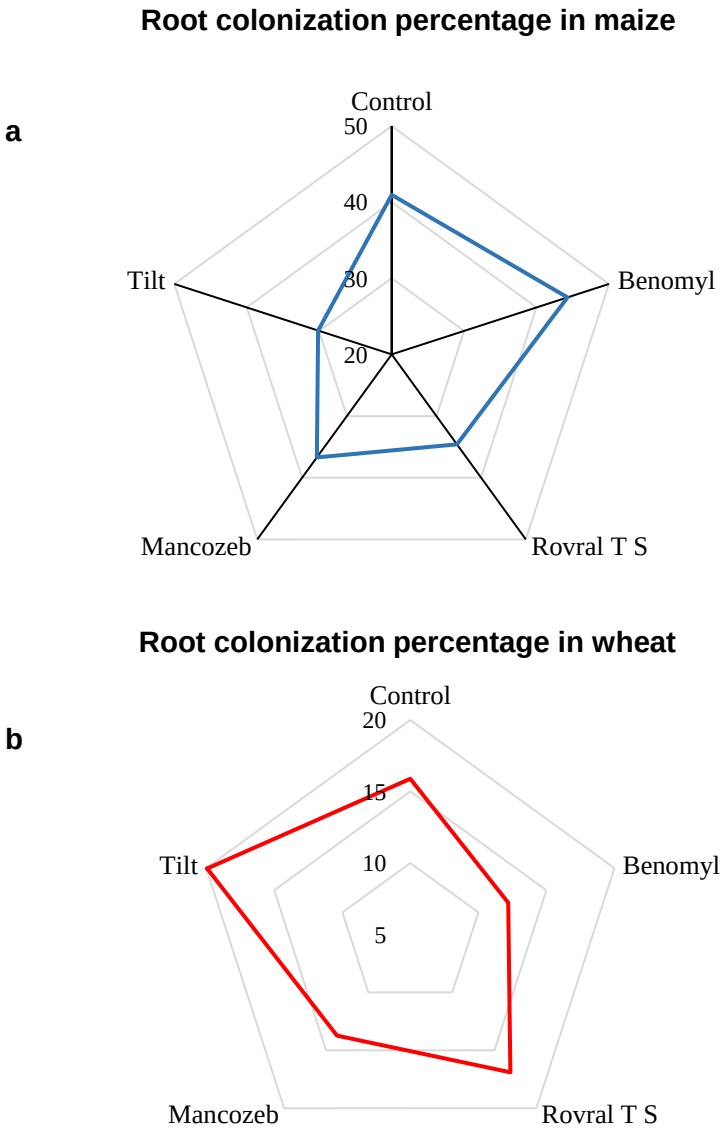

Figure 2. Radar charts for root colonization with *Rhizophagus irregularis* in a: maize and b: wheat.

Wheat plants

Analysis of variance showed that fungicide treatments had significant effects on plant height ($p \leq 0.01$), root fresh and dry weight of wheat plants ($p \leq 0.05$). Benomyl and mancozeb reduced root fresh weight; on the contrary, tilt increased this parameter compared to the control plant (Table 4). Root dry weight was the highest in rovral TS (Table 4), however, it did not reveal any significant differences with control.

Table 4. Mean comparison of fungicides treatments on wheat morphological traits and root colonization percentage.

Treatments	RF	RD	SF	SD	SL	Root colonization
	(g pot^{-1})				(cm)	(%)
Control	6.77 a	0.86 ab	14.39 a	2.75 ab	51.08 bc	15.89 a
Benomyl	4.96 b	0.59 b	11.67 b	2.27 b	46.01 c	12.20 a
Rovral TS	5.80 ab	1.05 a	13.99 a	2.33 ab	57.58 a	16.90 a
Mancozeb	4.50 b	0.58 b	13.51 ab	2.76 ab	59.35 a	13.73 a
Tilt	7.29 a	0.95 a	12.55 ab	2.96 a	55.30 ab	19.93 a

Different letter(s) showed significantly differences between treatments (Duncan $p \leq 0.05$).
RF: Root Fresh weight; RD: Root Dry weight; SF: Shoot fresh weight; SD: Shoot Dry weight; SL: Shoot Length.

Even though there were no significant differences between treatments but shoot fresh weights in wheat plants were reduced by fungicide application, benomyl specifically reduced the shoot fresh weight to 11.67 g in the pot while control treatment produced 14.39 g in the pot. Wheat height by the application of Benomyl was 46.01 cm, which in comparison with control (51.08 cm) reveals the negative impact of this fungicide on the wheat plant even though the difference was not significant. The longest plant height was observed with mancozeb application (Table 4). Although, there were no significant differ,

Laboratory experiment

Our result implies that in laboratory experiment, depending on the type of fungicide, the effect of the fungicides on root colonization percentage and spore number *R. irregularis* varies. It is showed that fungicides affect the AM symbiosis with the host plant in different manners including negatively, neutrally, and positively [43]. In this study, we showed that rovral TS and mancozeb can negatively and significantly affect the spore number and root colonization percentage by *R. irregularis*. Similarly, mancozeb reduced spore number and root colonization of *Rhizophagus fasciculatus*. The in a study, mancozeb, mefenoxam and azoxystrobin fungicides significantly reduced the root colonization percentage [44]. Reduced root colonization of *R. irregularis* in fungicide treatments (rovral TS and mancozeb) might be due to the sensitivity of *R. irregularis* to these fungicides. The phospholipid fatty acid (PLFA) results showed a significant reduction in the abundance of the 16:1ω5 AMF biomarker using fungicides [44]. Also, reported that the PLFA fraction of 16:1ω5 fatty acid originates from AMF mycelium [45]. This means the negative effect of fungicides on AMF biomarkers may have indirect preventive effects on mycelial growth [44]. The detrimental effects with other fungicides (i.e. propiconazole) on spore number of *R. irregularis* have also been reported [46].

Benomyl and tilt among fungicide treatments exerted a less negative impact on root colonization by *R. irregularis*, but led to a significant reduction in spore number. Although, in the controversy with our results, a study showed that the growth of *Cenococcum geophilum* inhibited strongly by benomyl application [1]. Kjøller and Rosendahl (2000) showed that benomyl at low application rate (1 mg g^{-1} soil) hindered fungal alkaline phosphatase activity in both internal and external hyphae [47]. It has been also revealed that benomyl constrained the spore germination and hyphal length of *G. mosseae* when applied at doses of 21.25 µg mL^{-1}, 10.62 µg mL^{-1} and 10 µg mL^{-1} [48]. The differences in relation to the effects of fungicides on spore number and root colonization percentage by *R. irregularis* could be attributed to the sensitivity of *R. irregularis* toward diverse fungicides applied as reported for *Glomus* species [47]. Channabasava and coauthors also reported different effects of Benomyl, Bavistin, Captan, and Mancozeb fungicides on the spore number and the root colonization percentage of *R. fasciculatus* and stated that this could be due to different susceptibility of *R. fasciculatus* to these fungicides [49].

Greenhouse experiment

Most of the fungicide treatments increased root dry weight in maize plants, among them benomyl showed significant difference with control. The application of fungicides to the soil may improve root growth [44, 49]. It is disclosed that mancozeb increased shoot and root dry biomass than all other fungicides and control and at a low dose (25–100 mg kg-1 soil) stimulated a higher rate of root growth [41]. In this study, the positive effect of fungicides especially benomyl on maize growth can be attributed to several factors including higher nitrogen uptake, inhibition of soil pathogens, or cytokinin-like effects of benomyl. The better performance of maize was directly or indirectly affected by benomyl due to the higher nitrogen absorption [50]. Benomyl contains 6% N, which directly degradable through microbial activity in the soil. The indirect effect of benomyl on the nitrogen content of the plant is through the alleviation of competition between AMF and plant for nitrogen, especially in nitrogen-limiting soils [50]. Püschel and coauthors claimed for the limited mycorrhiza benefits on *Andropogon gerardii* after plant and fungus compete for nitrogen under limited nitrogen supply [46].

The inhibitory effect of benomyl on soil pathogens in particular in non-sterilized soil could be another possible reason for better plant performance after the application of benomyl, the more reduced pathogen pressure the more growth stimulation by benomyl application [50]. In the current study, we also have applied non-sterilized soil. Hence, it would be a conceivable reason for maize better performance as colonization percentage was not diminished by benomyl application in maize. It has been also stated that benomyl may stimulate plant growth through having cytokinin-like effects, reducing leaf senescence, thereby improve plant biomass in some species [50]. Even though benomyl application had not shown any stimulatory effect on

wheat growth, but other researchers displayed the different effects of Topsin-M application, a fungicide with a similar mode of action as benomyl, on plant performance depending on plant species [51].

The type of fungicides applied in the soil must be considered because of the varied effect on the plant growth and AM fungal symbiosis [47]. A study showed that benomyl reduced mycorrhizal colonization of maize plants [50]. On the contrary, benomyl application in our study induced a higher root colonization percentage in the maize plant, though there was no significant difference with control. On the other hand, tilt reduced colonization percentage. A study showed that some fungicides (such as furadon and termix) at their recommended application rates reduced AM colonization and sporulation in three types of millet (*Eleusine coracana*, *Panicum miliaceum*, and *Paspalum scrobiculatum*) under field conditions, while others (including formaldehyde, bavistin, cuman, copperthom and sulfex) had no effect or even increased AM colonization and sporulation, depending on the species of cultivated millet [52]. In direct contrast with our results, Brundrett and coauthors stated that benomyl suppressed AM fungi spore production and seedlings AM infection [40]. Also, another study showed that colonization of *R. fasciculatus* with millet roots and its mycorrhizal spore number decreased by the application of benomyl (26% and 28.5%, respectively) and mancozeb (7.5 and 2.9%, respectively), whereas captan stimulated these characteristics by 13 and 12 percent [49]. Similar to our results, the total colonization of the chicory roots was significantly decreased in the presence of propiconazole by 59 and 40% at 0.2 and 2 mg L^{-1}, respectively [46].

Tilt application in wheat plants stimulated higher root colonization although differences with control were not significant. Propiconazole (Tilt 250 EC) at lower concentrations (0.1-1 ppm) stimulated the growth of some ectomycorrhizal fungi [1]. Generally, among the applied fungicide treatments, tilt and rovral TS could be recommended to use simultaneously with *R. irregularis* in wheat cultivation. The overall results suggest that the applied fungicides had no adverse impact on the activity and efficiency of *R. irregularis* inoculant in the pot experiments, hence we could be able to simultaneously use this biofertilizer with the applied fungicides in particular benomyl for maize and tilt for wheat production.

CONCLUSION

The applied fungicides in the laboratory experiment impaired root colonization percentage of *R. irregularis* and spore number, although in the greenhouse experiment there were no adverse effects even in some cases, used fungicides increased plant growth. The benomyl, mancozeb, rovral TS had no negative effects on the activity, and the efficiency of *R. irregularis* inoculant in maize cultivation thereby could be used safely with this bio-fertilizer, although, tilt and rovral TS were the safe fungicides for *R. irregularis* inoculant in wheat cultivation. Therefore, depending on the type of fungicide and the host plant, the effect of the fungicide on colonization and association of mycorrhiza varies.

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