PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 13 (12): 735-741, 2014
ISSN 1680-5194

Effect of Different Fungicides Application on Wheat Yield and Soil Native Status of Arbuscular Mycorrhizal Fungi

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Abstract: A field experiment was conducted to investigate the effect of fungicides on soil native status of Arbuscular mycorrhizal fungi (AMF) and yield of wheat grown in randomized complete block design with three replications during Rabi 2011-12. Three different fungicides commonly practiced by the farmers i-e Chloroflorochlorine, Acrobat and Redimoldgold were applied to crop. The recommended level of Chloroflorochlorine was 1 kg/ha, while that of Acrobat and Redimoldgold were 620 g/ha, respectively. Data revealed that wheat yield, plants nutrients uptakes and soil spores density of AMF and their roots infection intensity decreased with fungicides application as compared with the treatment applied with no fungicides. Highest reduction in grain yield shoot dry matter and plant height were noted with application of Acrobat 50% more than recommended level. Grain and shoot dry matter yields of 3621 and 9212 kg/ha, respectively, produced in this treatment, which 17% less than the treatment of no fungicides application. The application of acrobat 50% more than recommended level also showed decreases maximum in soil total N and AB-DTPA extractable P and Cu, which was significantly ($p \leq 0.05$), lower by 8 and 40%, respectively over the treatment of no fungicides application. Redimoldgold applied as 50% more than recommended level decreased more AB-DTPA extractable Zn and Mn contents lowered as 48 and 200%, respectively over the treatment of no fungicides application. The highest decreases in plant N and P uptake were observed by the application of Acrobat applied as 50% more than recommended level, lowered as 23 and 30%, respectively over the treatment of no fungicides application. Maximum decrease in spores density and their roots infection intensity were recorded by the application of Acrobat 50% more than recommended level, which were 67 and 50%, respectively, lower than the treatment of no fungicides application. Results suggest that application of fungicides reduces the efficiency of AMF native status in soil and cause reduction in wheat yield and plants nutrients uptake.

Key words: AMF spore density, infection intensity, Yield, Nutrients uptake, fungicides and wheat crop

INTRODUCTION

Mycorrhiza is the root fungi, which makes symbiotic association with roots of most crops and forest trees. Mycorrhiza may be ecto or endo mycorrhiza. Ectomycorrhiza are mainly present in the roots of forest trees, where as endomycorrhiza are commonly found in crops. Mycorrhiza enhances growth of the plants by increased absorption of water and nutrients from soil (Singh, 2003). Mycorrhizal fungi are beneficial symbiotic microorganisms with their extra matrical hyphae, which increase growth and yield of most crop plant through increase absorption of relatively immobile elements in soil such as P, Cu and Zn by substantially extending the area of absorption beyond that of root hairs (Nasim, 2005).

The use of different types of fungicides to control fungal diseases in crops is common practice in the area. Although, various fungal diseases in crops are controlled by these fungicides application but on the other hand, the application theses fungicides also affect the status of native AMF on large scale. The action of fungicides on AMF be related to a specific chemical derivative or formation. Hence, contact fungicides were reported to harm AMF development in the way that systemic fungicides can. There are only a few general trends of fungicidal actions on AMF, when applied to seeds (to control soil borne and seed borne pathogenic fungi which cause root and stem diseases. Fungicides apparently inhibit AMF infection more than when applied to foliage. The application of fungicides often retards the colonization of AMF. The effect of fungicide in most of time detrimental to AMF, but its degree of toxicity varies with application rate, active ingredient (Manjunath and Bagyaraj, 1984; Habate and Manjunath 1991; Salem *et al.*, 2003).

The application of systemic fungicides to soil near plants roots can absorbed by these roots and translocated to the plants other parts. These fungicides persistent in the crops and can affect the vegetative and reproduction of AMF. It is also reported that these fungicides affect symbiosis of AMF with roots and hence decreasing plants phosphorous uptake. Fungicides

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application often most detrimental, leading to decrease in indigenous fungal population and AMF infection intensity (Trappe *et al*., 1984). Beltanol and Previcur and also found that three fungicides of the foliar recommended application: Frupica, Rubigan, and Sinthane strongly inhibited AMF colonization, but Forum, Aliette, Swich, Teldor and Ortiva did not reduce it substantially. Narender (2011) found that the fungicides significantly inhibited the colonization of AMF. The highest reduction in AMF colonization was recorded in the treatment of application of mancozed followed by the treatments of copper sulphate and aureofungin, while there was lowest reduction of AMF colonization was observed in the carbendazim application.

MATERIALS AND METHODS

A field experiment was conducted in the research farm of The University of Agriculture, Peshawar to determine the effect of different fungicides application on the efficiency and status of native Arbuscular mycorrhizal fungi in wheat. Wheat (Siran, 2010) crop with seed rate of 120 kg/ha was grown in randomized complete block design (RCBD) with three replications. Three different fungicides commonly practiced by the farmers i.e., Chloroflorochlorine, Acrobat and Redimoldgold were applied to wheat crop in field. The recommended level of Chloroflorochlorine was 1 kg/ha, while that of Acrobat and Redimoldgold were 620 g/ha, respectively. All the three fungicides were applied in three split doses as per farmer's practices. The first dose was applied as soil application, while the remaining fungicides doses were applied as foliar spray on wheat crop with 30 days interval as 0.4% diluted solution of chloroflorochlorine and 0.25% of Acrobat and Redimoldgold uniformly on their corresponding treatments. The treatments combinations for the experiment were as Control (No fungicides application), Chloroflorochlorine (CFC) applied 50% less than recommended level, CFC applied at recommended level, CFC applied 50% more than recommended level, Acrobat applied 50% less than recommended level, Acrobat at recommended level, Acrobat 50% more than recommended level, Redimoldgold applied 50% less than recommended level, Redimoldgold at recommended level, Redimoldgold 50% more than recommended level.

Uniform Dose of N was applied at the rate of 120 kg N/ha in the form of Urea as three split applications. Phosphorous was applied as DAP at the rate of 45 kg P_2O_5/ha and K as potassium sulphate at the rate of 60 kg K/ha. All P and K fertilizers were applied at sowing time. The crop was harvested at maturity stage and all agronomic practices were strictly followed in all treatments uniformly throughout the growing season for optimum crop growth and yield production. Physicochemical characteristics of the soil under investigation were determined for texture by method as described by Koehler (1984), soil pH by procedure of McClean (1982), lime was determined by method as described by Richard (1954). For determination of soil organic matter content method of Nelson and Sommer (1982) was used. Soil total N was determined by Kjeldahl method of Bremner and Mulvanery (1996a). The P and micronutrients content were determined by method used by Soltanpour and Schwab (1977). The analysis of composite soil sample showed that textural class was silty clay loam and soil was alkaline in nature. The soil was low in total N less than 1%, O.M and AB-DTPA extractable P content. The soil was strongly calcareous in nature containing 19% lime.

Post harvest data regarding different parameters were recorded. These parameters included wheat grain yield, total dry matter yield, plant nutrients concentration and their uptake, The AMF soil spores density and their root infection intensity. Total dry matter yield was recorded in each treatment after drying. Plants samples were digested and analyzed according to the procedure as described by Walsh and Beaton (1977). Plants N and P concentrations of wheat were determined. To avoid the effect of dilutions or concentrations caused by variation in wheat plants yield, the nutrients concentration were converted into total amount of plant nutrients uptake plants by multiplying nutrient concentrations with total dry matter yield in kg/ha by the procedure as described by Nye and Tinker (1977), Jarrell and Beverly (1981) and Barber (1984). Fresh soil and roots samples were also collected for the determination of spores density and AMF root infection intensity. Plants, N concentration were determined by Kjeldahl method (Ryan *et al*., 2002) and plants, P concentration by Wet-Digestion Method (Jackson, 1973). Phosphorous was measured using Lambda 35 spectrophotometer. The AMF spores were isolated from soil by wet-sieving and decanting techniques as described by Bremner and Mulvaney (1996b). Infection intensity of AMF in wheat roots was determined according to the procedures of Philips and Hayman (1970) and Koske and Gemma (1989). The presences of vesicles, arbuscules were measured by the techniques as described by Giovannetti and Mosse (1980). Spores isolated from the soil samples were identified according to their morphological characteristics including size, shape, color, distinct wall layer, attached hyphae and surface orientation of spores as described by Schenck and Perez (1990).

Statistical analysis of data: Statistical analysis of data was carried out by conducting ANOVA and the means were compared by LSD test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Field experiment was conducted in research to investigate the effect of different fungicides application on AMF spores density in soil, their root infection intensity and yield, N and P uptakes of wheat crop.

Wheat yield and yield components: Wheat yield and yield components as affected by the application of different fungicides are presented in the Table 1.

It is evident from the data in Table 1 that application of different fungicides have decling effects on the yield and yield components of wheat. The data revealed that maximum wheat grain yield of 4221 kg/ha was recorded in the treatment of N, P and K fertilizers applied with no fungicide of any kind. Minimum grain yield of 3621 kg/ha was found in the treatment of Acrobat 50% more than recommend level, which was 17% less than the treatment of no fungicides application (Fig. 1). The data indicated that maximum shoots dry matter yield of 10750 kg/ha was observed in the treatment of N, P and K fertilizers with no fungicides application and minimum shoot dry matter yield of 9212 kg/ha was noted in the treatment of Acrobat 50% more than recommended level and was 17% less than the treatment of no fungicides (Fig. 2). The highest straw yield of 6529 Kg/ha was found in the treatment of no fungicides and minimum of 5123 kg/ha in the CFC 50% less than recommended level, which was 27% less than the treatment with no fungicides (Fig. 3). Data indicated that highest plants height of 88.2 cm was recorded in the treatment of N, P and K fertilizers with no fungicides and lowest of 85cm in the treatments of Acrobat recommended level and 50% more than recommended level and was 4% less than the treatment with no fungicides.

The data show that maximum spike length of 11 cm was found in the treatment with no fungicides and minimum of 10.2 cm in the treatment of Redimoldgold 50% more than recommended level and 8% less than the treatment with no fungicides. It is evident from the data that relatively lower yield and yield components of wheat crop were recorded with the application of fungicides at different levels. Assaf *et al*. (2009) reported that soil fungicide treatment significantly decreased crop biomass and grain yield as compared to no fungicides application. Result showed that biomass of chickpea in treated soil with fungicide was 5162 kg/ha as compared to no fungicides application which was 5707 kg/ha similarly seed yield in treated soil was 2260 kg/ha as compare to no fungicides application which was of 2295 kg/ha. Sukarno *et al*. (1993) reported that when metalaxyl was applied in onion as a soil drench it reduced plant growth. Darwesh and Mustafa (2012) found that highest yield in the treatment of fungicides application in lower concentration and lowest yield with application of fungicides in higher concentration. These results support our study that in most cases the fungicides effect the plant growth.

Post harvest soil nutrients contents: Post harvest soil total N and AB-DTPA extractable P, Zn, Cu, Mn and Fe contents as affected by the application of different fungicides are presented in Table 2.

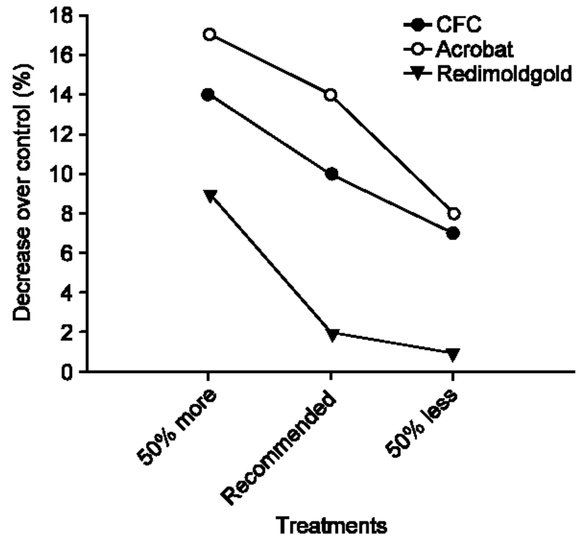

Fig. 1: Percent decrease in grain yield with fungicides in relation to no fungicides application

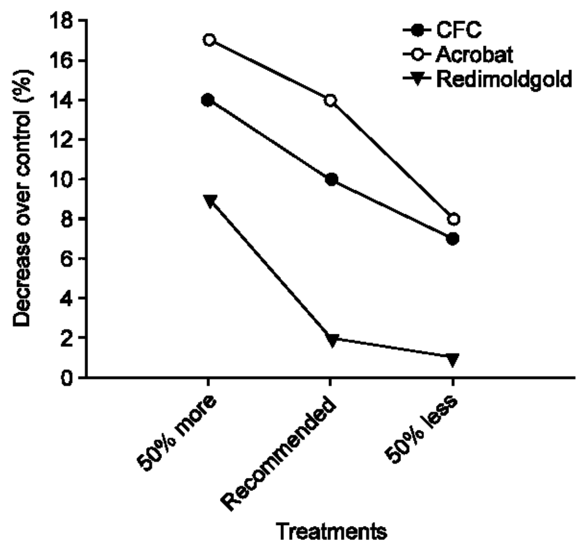

Fig. 2: Percent decrease in shoot dry matter with fungicides in relation to no fungicides application

The data indicated that maximum post harvest soil total N content of 1163 mg/kg was found in the treatment of N, P and K fertilizers with no fungicides application and in the treatment Redimoldgold 50% less than recommended level. Minimum soil N content of 1080 mg/kg was observed in the treatment of Acrobat 50% more than recommended level, which was significantly ($p \leq 0.05$), decreased as 8% over the treatment of no fungicides application and Redimoldgold 50% less than recommended level. The data indicated that maximum post harvest soil AB-DTPA extractable P of 5.51 was recorded in the treatment of N, P and K fertilizers applied with no fungicides and minimum of 5 mg/kg in the

Table 1: Grain, shoots dry matter and straw yields, plant height and spike length of wheat as affected by different fungicides application

	Grains	Shoot dry matter	Straw	Plant height	Spike length
Treatments	---------------------- (yield kg/ha)	----------------------		------------------ cm	------------------
N, P and K fertilizers (No fungicides)	4221^{a*}	10750^{a*}	6529^{a*}	88.20^{a*}	11.00^{a*}
Chloroflorochlorine (CFC) 50% less	3940^{b}	10063^{c}	6123^{b}	87.10^{b}	10.96^{a}
CFC at recommended level	3818^{f}	9735^{d}	5557^{c}	86.00^{c}	10.20^{d}
CFC 50% more	3696^{d}	9423^{e}	5727^{c}	85.10^{d}	10.85^{b}
Acrobat 50% less	3891^{bc}	9945^{cd}	6054^{b}	85.46^{cd}	10.96^{a}
Acrobat at recommended level	3708^{d}	9425^{e}	5717^{c}	85.00^{d}	10.96^{a}
Acrobat 50% more	3621^{d}	9212^{e}	5591^{c}	85.00^{d}	10.80^{b}
Redimoldgold 50% less	4181^{a}	10677^{ab}	6496^{a}	87.10^{b}	10.65^{c}
Redimoldgold at recommended level	4127^{a}	10515^{b}	6388^{a}	85.53^{cd}	10.80^{b}
Redimoldgold 50% more	3872^{bc}	9840^{d}	5968^{b}	87.05^{b}	10.16^{d}

*Means with different letter (s) in columns are significantly different at $p \leq 0.05$ N-P-K @ 120-45-60 kg/ha, respectively, recommended dose CFC = 2.5 kg/ha, Acrobat and Redimoldgold = 0.6 kg/ha

Table 2: Post harvest soil nutrients contents as affected by application of different fungicides

		---------- AB-DTPA extractable	----------	----------	----------
		P	Zn	Cu	Mn	Fe
Treatments	Total N	---------- Soil contents (mg/kg)	----------	----------	----------	----------
N, P and K fertilizers (No fungicides)	1163^{a}	5.51^{a*}	1.08^{a*}	3.03^{a*}	4.11^{a*}	2.63^{a*}
Chloroflorochlorine (CFC) 50% less	1133^{ab}	5.33^{abc}	0.92^{abc}	2.21^{c}	2.89^{abc}	1.70^{b}
CFC at recommended level	1133^{ab}	5.10^{bc}	0.97^{ab}	2.31^{c}	3.07^{ab}	2.35^{ab}
CFC 50% more	1112^{bc}	5.0^{c}	0.81^{bc}	2.21^{c}	2.44^{bc}	1.81^{b}
Acrobat 50% less	1112^{bc}	5.24^{abc}	1.07^{a}	2.18^{c}	3.42^{ab}	2.35^{ab}
Acrobat at recommended level	1133^{ab}	5.03^{c}	0.76^{bc}	2.31^{c}	3.30^{ab}	1.96^{ab}
Acrobat 50% more	1080^{c}	5.0^{c}	0.83^{bc}	2.33^{c}	3.33^{ab}	2.28^{ab}
Redimoldgold 50% less	1163^{a}	5.40^{ab}	0.74^{c}	3.20^{a}	3.57^{ab}	2.58^{a}
Redimoldgold at recommended level	1146^{ab}	5.33^{abc}	0.75^{c}	2.66^{b}	3.48^{ab}	2.58^{a}
Redimoldgold 50% more	1112^{bc}	5.06^{bc}	0.73^{c}	2.41^{bc}	1.46^{c}	2.16^{ab}

*Means with different letter (s) in columns are significantly different at $p \leq 0.05$ N-P-K @120-45-60 kg/ha, respectively, recommended dose CFC = 2.5 kg/ha, Acrobat and Redimoldgold = 0.6 kg/ha

Table 3: Plants N and P uptakes of wheat as affected by fungicides

	N	P
Treatments	Plants uptakes (kg/ha)	
N, P and K fertilizers (No fungicides)	187^{a*}	18.3^{a*}
Chloroflorochlorine (CFC)50% less	169^{b}	16.3^{c}
CFC at recommended level	167^{b}	15.0^{e}
CFC 50% more	158^{cd}	14.1^{f}
Acrobat 50% less	170^{b}	15.7^{d}
Acrobat at recommended level	167^{b}	14.9^{e}
Acrobat 50% more	152^{d}	14.1^{f}
Redimoldgold 50% less	182^{a}	17.7^{b}
Redimoldgold at recommended level	182^{a}	17.6^{b}
Redimoldgold 50% more	167^{b}	15.9^{d}

*Means with different letter(s) in columns are significantly different at $p \leq 0.05$ N-P-K @120-45-60 kg/ha, respectively, recommended dose CFC = 2.5 kg/ha, Acrobat and Redimoldgold = 0.6 kg/ha

treatment of Acrobat 50% more than recommended level and was 105% less than the treatment with no fungicides application. The data revealed that maximum soil Zn content of 1.08 mg/kg was found in the treatment of N, P and K fertilizers with no fungicides application and minimum of 0.73 mg/kg in the treatment of Redimoldgold 50% more than recommended level, which was decreased as 48% over the treatment with no fungicides application. Maximum soil Cu content of 3.03 mg/kg was recorded in the treatment with no fungicides application and minimum of 2.18 mg/kg in treatment of Acrobat 50% less than recommended level and was 40% less than the treatment with no fungicides application.

It is evident from the data that maximum soil Mn content of 4.11 mg/kg was found in the treatment of no fungicides application and minimum of 1.46 mg/kg in the treatment of Redimoldgold 50% more than recommended level, which was 200% decreased over the treatment with no fungicides application. The data revealed that maximum soil Fe content of 2.63 mg/kg observed in the treatment of N, P and fertilizers applied no fungicides and minimum of 1.70 mg/kg in the treatment CFC 50% less than recommended level and was 55% less than the treatment with no fungicides application.

Plants N and P uptakes: Data on plants N and P uptakes by wheat as affected by different fungicides application are presented in Table 3. The data indicated maximum plants N uptake of 187 kg/ha was found in the treatment with no fungicides application and minimum of 152 kg/ha in the treatment of Acrobat 50% more than recommended level, which was decreased by 23% over the treatment with no fungicides application (Fig. 3). Maximum plants P uptake of 18.3 kg/ha was observed in the treatment of N, P and K fertilizers applied with no

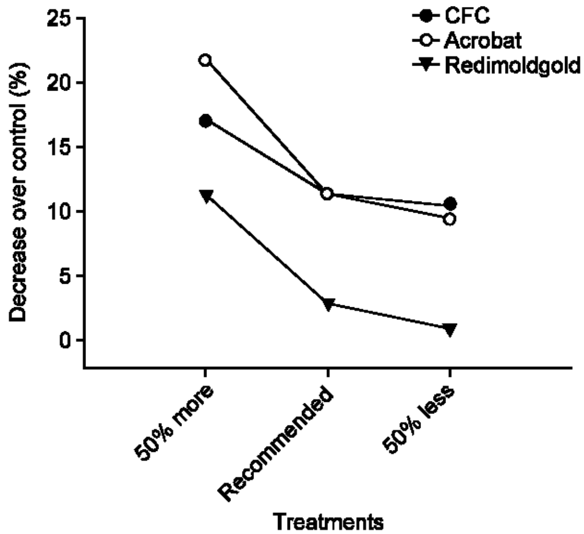

Fig. 3: Percent decrease in N uptake with fungicides in relation to no fungicides application

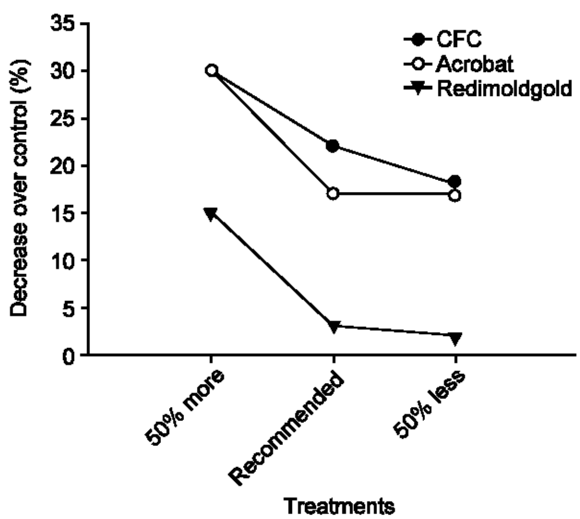

Fig. 4: Percent decrease in P uptake with fungicides in relation to no fungicides application

fungicides and minimum of 14.1 kg/ha in the treatments CFC 50% than recommended level and Acrobat 50% more than recommended level and was 30% less than the treatment with no fungicides application (Fig. 4). Darwesh and Mustafa (2012) found that N and P uptakes were maximum in the treatments of application of fungicides in lower concentrations than the treatments of higher concentrations.

Soil spores density and root infection intensity of AMF: Soil spores density of AMF and their roots infection intensity of wheat crop as affected by different fungicides are presented in Table 4.

It is evident from the data that maximum spores density of 16/20 g soil was observed in the treatment of N, P and K fertilizers applied with no fungicides and minimum of 9/20 g soil in the treatment of Acrobat 50% more than recommended level, which significantly ($p \leq 0.05$) decreased as 67% over the treatment with no fungicides application. The data indicated that highest AMF root infection intensity of 17% was recorded in the treatment with no fungicides application and minimum of 11% in the treatments in CFC 50% more than recommended level and Acrobat 50% more than recommended level and was 55% less than the treatment with no fungicides application.

Sukarno *et al.* (1993) found that when metalaxyl was applied in onion as a soil drench it reduced number of arbuscules and length of infected roots. Other fungicides such captan, benomyl, emisan and pentachloronitrobenzene have negative effect when applied as soil drenches on AMF colonization. Schreiner and Bethlenfalvay (1997) and Kjoller and Rosendah (2000) found that fungicides restricted the development of AMF. Assaf *et al.* (2009) investigated that soil treated with fungicide slightly decreased AMF root infection intensity rate over the treatment with no fungicides application. Narender (2011) found that the fungicides significantly inhibited the colonization of AMF. These results support our experiment result that fungicide decreased AMF spores density and AMF root infection intensity. The isolated AMF spores were identified by the method as described by Schenck and Perez (1990).

The use of different types of fungicides to control fungal diseases in crops is common practice in the area. Although, various fungal diseases in crops are controlled by these fungicides application but on the other hand, the application theses fungicides also affect the status of native AMF on large scale. The action of fungicides on AMF be related to a specific chemical derivative or formation. Hence, contact fungicides were reported to harm AMF development in the way that systemic fungicides can. There are only a few general trends of fungicidal actions on AMF, when applied to seeds (to control soil borne and seed borne pathogenic fungi which cause root and stem diseases. Fungicides apparently inhibit AMF infection more than when applied to foliage. The application of fungicides often retards the colonization of AMF. The effect of fungicide in most of time detrimental to AMF, but its degree of toxicity varies with application rate, active ingredient (Manjunath and Bagyaraj, 1984; Habate and Manjunath, 1991; Salem *et al*., 2003). The application of systemic fungicides to soil near plants roots can absorbed by these roots and translocated to the plants other parts. These fungicides persistent in the crops and can affect the vegetative and reproduction of AMF. It is also reported that these fungicides affect symbiosis of AMF with roots and hence decreasing plants P uptake. Systemic fungicides which are commonly used in agriculture Ditiver, Octagon, Metaram and Parmex eliminated the AMF symbiosis in

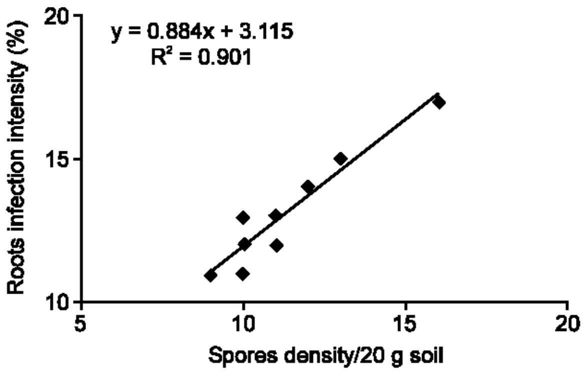

Fig. 5: Relationships between soil spores density of AMF and their roots infection intensity in wheat

Table 4: Spores density and root infection intensity of AMF as affected by application of different fungicides

Treatments	AMF spores density (20 g soil)	A AMF root infection intensity (%)
Control	16^{a*}	17^{a*}
Chloroflorochlorine 50% less	12^{bc}	14^{bc}
Chloroflorochlorine at recommended level	10de	13^{cd}
Chloroflorochlorine 50% more	10de	11^{e}
Acrobat 50% less	10de	12de
Acrobat at recommended level	11^{cd}	12de
Acrobat 50% more	9^{e}	11^{e}
Redimoldgold 50% less	13^{b}	15^{b}
Redimoldgold at recommended level	12^{bc}	14^{bc}
Redimoldgold 50% more	11^{cd}	13^{cd}

*Means with different letter (s) in columns are significantly different at $p \leq 0.05$ N-P-K=120-45-60 kg/ha, respectively, Chloroflorochlorine = 2.5 kg/ha, Acrobat = 0.6 kg/ha, Redimoldgold = 0.6 kg/ha

the treated plants, while AMF colonization was not affected by the soil treatment with INACOP. Fungicides application often most detrimental and hence decrease indigenous fungal population and AMF infection intensity (Trappe *et al*., 1984). Narender (2011) found that the fungicides significantly inhibited the colonization of AMF. The highest reduction in AMF colonization was recorded in the treatment of application of mancozed followed by the treatments of copper sulphate and aureofungin, while there was lowest reduction f AMF colonization was observed in the carbendazim application.

Conclusion: It was concluded from the results of experiment that application of fungicides (Chloroflorochlorine, Acrobat and Redimoldgold) in normal soil caused reduction in soil spores density and their root infection intensity in wheat crop. Wheat yield, yield components and plant nutrients uptake decreased by the application of different fungicides as compared with the treatment applied with no fungicides. Highest reduction in wheat yield, plant nutrients uptake and AMF status were observed with application of Acrobat as compared with other two fungicides. Further research work is needed in this area to investigate different other fungicides affects on different crops and soil AMF status.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Higher Education Commission of Pakistan for financial support of this study.

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