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A Comparative Analysis of Tebuconazole Mediated Phytotoxicity to Legumes

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ABSTRACT

In agricultural operations, fungicides are used to protect the crop plants from fungal pathogens. An indiscriminate and injudicious application of fungicides leads to their accumulation in upper soil layers of agricultural fields and exerts damaging impact on soil fertility as well as the crop productivity. The present study was therefore, navigated to assess the effect of triazole fungicide tebuconazole concurrently on common food legumes (chickpea, pea, lentil and greengram). The recommended field rate of the technical grade (active ingredient 100% w/w) tebuconazole was used in soils. Root and shoot biomass, chlorophyll content, nodule number, nodule biomass, leghaemoglobin content, seed protein and seed yields of each legume were measured at an appropriate time following different methods. The recommended field rate of tebuconazole showed a varying degree of phytotoxicity to the tested legumes. Tebuconazole significantly ($p \leq 0.05$) decreased shoot growth, leghaemoglobin, chlorophyll, shoot N, shoot P and seed protein in chickpea to the highest level. Generally, tebuconazole showed the most adverse effect on the growth parameters of chickpea and affected the least those of greengram. These findings demonstrated that an outcome of the effects of a specific fungicide on a specific crop plant species can not be generalized. The degree of toxicity of any fungicides and the type of plant organs affected may differ from one plant species to another.

Key words: Fungicide, legume, leghaemoglobin, soils, tebuconazole, toxicity

INTRODUCTION

With the sole objective of optimization of crop productivity and economic return, application of mineral fertilizers, bio-inoculants, organic amendments and pesticides in soils are some of the frequently employed strategies in current agricultural operations (Parvez *et al.*, 2003; Gaind *et al.*, 2007; Ismail *et al.*, 2009; Ahemad and Khan, 2011a). Intensive applications of agrochemicals mainly pesticides have become a major concern due to various ill-effects on natural environments (El-Shenawy *et al.*, 2003; Pal *et al.*, 2006; Abou Ayana *et al.*, 2011) and have stimulated scientists to assess their effects on different ecosystems. Among pesticides, one or more fungicides of several chemical groups are frequently applied to seeds to protect crops from soil borne fungal pathogens. A major fraction of these plant protection agents especially the pesticides accumulates into soils because of their continual application and decreases the metabolic activities of soil microflora leading to the losses in soil fertility (Abdalla and Langer, 2009; Ahemad and Khan, 2011b, c). Pesticides including fungicides are reported to reduce the nodulation, the nitrogenase activity, the legume-*Rhizobium* symbiosis in legumes (Dunfield *et al.*, 2000) and affect plant growth promoting traits of rhizobacteria (Ahemad and Khan, 2010a) and soil characteristics (Nare *et al.*, 2010).

Consequently, it is particularly important to test the toxicity of the recommended pesticides for specific crops as precisely as possible.

Conazole fungicides including tebuconazole (broad spectrum, systemic, triazole fungicide) disrupt the membrane functions by inhibiting the sterol biosynthesis in phytopathogenic fungi (Ahemad and Khan, 2010b). In India, triazole fungicides including tebuconazole are used extensively against powdery mildew, loose smut and rust of both legume and non-legume crops (Kishorekumar *et al.*, 2007; Singh and Dureja, 2009; Mohapatra *et al.*, 2010). Most of the earlier studies of phyto-toxicity of fungicides are generally, restricted to any single crop and the comprehensive data assessing the impact of any specific fungicide on more than one legume in parallel is rare. Hence, the present study was designed to evaluate the effect of tebuconazole on four commonly grown legumes like, chickpea (*Cicer arietinum* L.), pea (*Pisum sativum*), lentil (*Lens esculentus*) and greengram (*Vigna radiata* L. Wilclzek) simultaneously so that a firm conclusion can be drawn about the effects of tebuconazole on legumes.

MATERIALS AND METHODS

The experiments were conducted for two consecutive years (2007-2008 and 2008-2009) with the identical environmental conditions and with the same fungicide treatments to ensure the reproducibility of the results.

Fungicide treatment: The technical grade (active ingredient 100% w/w) tebuconazole was obtained from Parijat Agrochemicals (New Delhi, India). To prevent the degradation, the stock solution was prepared just prior to each experiment by dissolving fungicide in solvent [dimethyl sulfoxide (DMSO)]. The recommended field dose (100 $\mu g\ kg^{-1}$ soil) of tebuconazole was used for the experiments.

Legume growth measurement: Seeds of commonly grown legumes like, chickpea var. C235, pea var. arakle, lentil var. K75 and greengram var. K851 were obtained from Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India. Seeds of these legumes were surface sterilized with 70% ethanol, 3 min.; 3% sodium hypochlorite, 3 min; rinsed six times with sterile water and dried. The sandy clay loam soils used for the pot experiments had the following physical and chemical properties: organic carbon (C) 0.4 %, Kjeldahl nitrogen (N) 0.75 $g\ kg^{-1}$, Olsen phosphorus (P) 16 $mg\ kg^{-1}$, pH 7.2 and water holding capacity 0.44 $mL\ g^{-1}$, cation exchange capacity 11.7 $cmol\ kg^{-1}$and 5.1 $cmol\ kg^{-1}$ anion exchange capacity. A total of ten seeds of each legume were sown in clay pots (25 cm high, 22 cm internal diameter) using three kg unsterilized soils. A control (without tebuconazole) and a treatment with the recommended field rate of tebuconazole were used in three replicates for each legume. Seeds of chickpea, lentil, greengram and pea were sown in October (2007), November (2007), March (2008) and November (2007), respectively. Plants in each pot were thinned to three plants 10, 10, 7 and 7 Days After Sowing (DAS) of chickpea, lentil, greengram and pea, respectively. The pots were watered with tap water when required and were maintained in an open field.

Biomass production and symbiotic attributes: All plants for each treatment were removed at 135 DAS (at harvest stage) of chickpea, 120 DAS (at harvest stage) for both pea and lentil and 80 DAS (at harvest stage) for greengram. The root and shoot of each legume were carefully washed and oven dried at 80°C and weighed. The nodulation in chickpea, pea and lentil was recorded at 90 DAS (pod fill stage) and that of greengram at 50 DAS (pod fill stage). Nodules from the root systems of each legume were separated, counted, oven dried at 80°C and weighed. The leghaemoglobin (Lb) content in fresh nodules recovered from the root system of each legume crop was quantified at 90 DAS each for chickpea and pea and lentil and 50 DAS for greengram, respectively by the method of Sadasivam and Manickam (1992).

Total chlorophyll, nitrogen and phosphorus contents: The total chlorophyll content in fresh foliage of each experimental legume crop was quantified at 90 DAS each for chickpea, pea and lentil and 50 DAS for greengram by the method of Arnon (1949). The total N and P content in roots and shoots of chickpea (135 DAS), lentil (120 DAS), pea (120 DAS) and greengram (80 DAS) were measured by the micro-Kjeldahl method of Iswaran and Marwah (1980) and the method of Jackson (1967), respectively.

Seed yield and grain protein: Chickpea, pea, lentil and greengram were finally harvested at 135, 120, 120 and 80 DAS, respectively and seed yield was measured. The protein content in grains of each legume was estimated by the method of Lowery *et al.* (1951).

To check the efficacy, the pot experiments were repeated the next year (2008-2009).

Statistical analysis: Since the data of the measured parameters obtained were homogenous, they were pooled together and subjected to analysis of variance. The difference among treatment means was compared by Tukey test at 5% probability level by statistical software, SPSS 10.

RESULTS AND DISCUSSION

The present study dealt with the parallel assessment of the growth parameters of the selected food legumes (chickpea, pea, greengram and lentil) in the presence of tebuconazole-stress (at the recommended field rate). A substantial variation was observed in the plant biomass and the symbiotic attributes of the tested legumes grown in tebuconazole-amended soils.

In present study, tebuconazole generally, showed a significant reducing effect on root and shoot growth of the selected legumes. Pea suffered the maximum reduction in root biomass among the selected legumes under tebuconazole-stress. Tebuconazole significantly ($p \leq 0.05$) decreased the root biomass of pea by 41%, over the untreated control. On the other hand, the least decline in root biomass (21%) was observed in greengram compared to control. Moreover, tebuconazole-mediated reduction (percent decline above control) in the root biomass of the legumes was observed in the following order: pea (41)>lentil (33)>chickpea (29>greengram (21). Contrary to the trend in root biomass reduction in the presence of triazole fungicide, the shoot biomass of the tested legumes decreased significantly ($p \leq 0.05$) in the following array: chickpea (53)>greengram (36)>pea (31)> lentil (24) (Table 1).

Further, the nodulation attributes of the legumes also varied substantially in the presence of the tebuconazole-stress. Generally, the recommended dose of tebuconazole displayed the negative impact on the nodule parameters for each legume. Of the selected legumes, the nodule development in pea was hampered by the highest degree (67% reduction in nodule numbers) over control. In contrast, tebuconazole suppressed significantly ($p \leq 0.05$) the nodule formation in other legumes approximately by similar level (at an average 23%, compared to their respective controls). Comparative evaluation of nodule numbers of legume species does not provide an accurate assessment because the size of nodules varies from one legume species to another. Both nodule dry biomass and the most importantly their Lb content are the precise parameters to assess the actual

Table 1: Effect of tebuconazole on dry biomass and symbiotic properties of legume crops

Legumes	Dose rate ($\mu g\ kg^{-1}$ soil)	Dry biomass ($g\ plant^{-1}$) Root	Shoot	Nodulation No./plant	Nodule biomass ($mg\ plant^{-1}$)	Leghaemoglobin content [$mM (g.f.m.)^{-1}$]
Chickpea	0 (control)	0.91[a]	3.80[a]	21[b]	180[b]	0.13[b]
	100	0.65[b]	1.80[d]	16[d]	116[c]	0.04[e]
Pea	0 (control)	0.92[a]	2.07[b]	27[a]	283[a]	0.17[a]
	100	0.54[c]	1.42f	9[e]	176[b]	0.12[c]
Greengram	0 (control)	0.47[d]	2.08[b]	21[b]	66[d]	0.08[c]
	100	0.37[e]	1.34[g]	16[d]	50[e]	0.07[d]
Lentil	0 (control)	0.55[c]	1.97[c]	19[c]	30f	0.12[c]
	100	0.37[e]	1.50[e]	15[d]	14[g]	0.08[c]
LSD ($p\leq0.05$)		0.04	0.06	001.8	07.25	0.005
F-value		26.90	220.60	267.4	157.30	77.80

Values are Mean of three replicates where each replicate constituted three plants/pot. Mean values followed by different letters are significantly different within a row or column at $p\leq0.05$ according to Tukey test; $(g.f.m.)^{-1}$ = $(\text{gram fresh biomass})^{-1}$

impact of any stress factor on nodulation (Wani *et al*., 2007). Therefore, these two symbiotic characteristics for each legume were also determined. The toxic effect of tebuconazole on dry biomass (percent decline over controls) was observed as-lentil (53)>pea (38)>chickpea (36)>greengram (24). Quite the opposite, tebuconazole decreased significantly ($p\leq0.05$) Lb content in nodules of the tested legumes in the following order (percent decline over respective controls): chickpea (69)>lentil (33)>pea (29)>greengram (12) (Table 1).

In this study, triazole fungicide tebuconazole negatively affected the growth of all tested legumes. The decline in legume growth with tebuconazole could be due to the toxic effects of this fungicide on the legume-*Rhizobium* symbiosis and N_2-fixation (Evans *et al*., 1991). In addition, the inhibitory effect of the fungicide application may possibly be due to the inhibition of enzymes involved in growth and metabolisms (Zablotowicz and Reddy, 2004) or due to disruption of signaling between (legume derived) phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors that is necessary for initiation of nodulation and N_2 fixation (Fox *et al*., 2007). In other studies, Kyei-Boahen *et al*. (2001) reported decreased nodulation, percent N derived from the atmosphere (% Ndfa) and plant growth in chickpea (*Cicer arietinum*) in response to pre-sowing seed treatment with commercial fungicides. However, the degree of toxicity of tebuconazole to the parameters to each legume varied considerably in our study. The variable response of the tested legumes to tebuconazole is due to the fact that extent of toxicity of any specific pesticide to the plants depends upon the both genetics and physiology of plants which varies from one plant species to another (Ahemad and Khan, 2011a).

In general, the chlorophyll content in leaves of each legume significantly decreased while grown in tebuconazole-applied soils. The most toxic impact of triazole fungicide on biosynthesis of chlorophyll was observed in chickpea plants wherein the chlorophyll reduced significantly ($p\leq0.05$) to 17% compared to control. Moreover, tebuconazole mediated reduction in the chlorophyll content of pea was the least (4% compared to control). The suppressive effect of tebuconazole on chlorophyll synthesis (percent reduction over controls) of the selected legumes followed the trend as- chickpea (17)>lentil (12)>greengram (9)>pea (4) (Table 2). As reported by Boldt and Jacobsen (1998) that the pesticides adversely affect the metabolic enzymes, therefore, it seems probable that fungicide employed in this study might have inhibited the functioning of the enzymes of Photosynthetic

Table 2: Effect of tebuconazole on biological and chemical properties of legume crops

Legumes	Dose rate ($\mu g\ kg^{-1}$ soil)	Chlorophyll content ($mg\ g^{-1}$)	N content ($mg\ g^{-1}$) Root	N content ($mg\ g^{-1}$) Shoot	P content ($mg\ g^{-1}$) Root	P content ($mg\ g^{-1}$) Shoot	Seed protein ($mg\ g^{-1}$)	Seed yield ($g\ plant^{-1}$)
Chickpea	0 (control)	1.96^a	18^d	27^d	00.17^d	0.21^e	241^b	2.7^e
	100	1.62^b	15^e	21^e	00.15^e	0.17f	209^e	1.7^f
Pea	0 (control)	0.75^d	34^b	45^b	00.21^b	0.28^c	224^d	7.4^a
	100	0.72^d	30^c	41^c	00.18^c	0.24^d	221^d	6.8^b
Greengram	0 (control)	0.82^c	36^a	50^a	00.27^a	0.36^a	261^a	7.4^a
	100	0.75^d	29^c	41^c	00.21^b	0.29^b	246^b	4.8^c
Lentil	0 (control)	0.32^e	17^d	45^b	00.21^b	0.28^c	232^c	3.0^d
	100	0.28^f	13^f	39^c	00.17^d	0.24^d	223^d	1.2^g
LSD ($p \leq 0.05$)		0.036	01.2	002.2	0.005	0.007	07.4	0.14
F-value		45.6	387.6	422.2	85.3	174.2	109.5	251.1

Values are Mean of three replicates where each replicate constituted three plants/pot. Mean values followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test

Carbon Reduction (PSCR) cycle, such as Rubisco, 3-PGA kinase, NADP, NAD-Glyceraldehyde-3-P-dehydrogenase and aldolase.

Tebuconazole decreased significantly ($p \leq 0.05$) the root N in chickpea, pea, greengram and lentil by 17, 12, 19 and 24%, respectively over respective controls. On the other hand, the reduction in the shoot N of each legume exposed to the fungicide-stress compared to the respective controls followed the trend as: chickpea (22%)>greengram (18%)>lentil (13%)>pea (9%) (Table 2). Furthermore, the root P of the tested legumes in the presence of tebuconazole-stress decreased significantly ($p \leq 0.05$) in the following order: greengram (22%)>lentil (19%)>pea (14%)>chickpea (12%) while tebuconazole decreased significantly ($p \leq 0.05$) shoot P over controls in an array: chickpea (19%) = greengram (19%)>pea (14%) = lentil (14%) (Table 2).

In present study, tebuconazole also decreased the seed protein in chickpea by 13%, compared to control whereas protein content in seeds of pea, greengram and lentil was marginally affected in the presence of fungicide. Generally, seed yield of each legume substantially decreased under tebuconazole-stress. The lentil suffered a significant ($p \leq 0.05$) decline in seed yield while the least decline was observed for that of pea in the presence of tebuconazole. The following decreasing trend (percent decline over controls) for seed yields was noted: lentil (60)>chickpea (37)>greengram (35)>pea (8) (Table 2).

Nitrogen and Phosphorus content of the legume plants is one of the most important aspects of legume growth. The nitrogen content in roots and shoots determined at different stages of chickpea, lentil, pea and greengram differed among treatments. The decrease in N contents of legumes might have been due to the reduction in legume-*Rhizobium* symbiosis, as indicated by a decline in the nodulation in this study. In agreement to this finding, Fox *et al*. (2007) concluded that agrichemicals induced a symbiotic phenotype that inhibited or delayed recruitment of rhizobia to host plant roots. Due to the decreased nodulation efficiency of rhizobia, fewer root nodules were produced. Therefore, the rate of nitrogenase activity was declined. In turn, both N content and the overall plant yields were decreased. However, the reduction in P content and seed attributes following the agrochemical application could probably, be due to inhibition of the enzymes and functional proteins of metabolic pathways involved in protein synthesis and P-uptake (Boldt and Jacobsen, 1998; Ahmad *et al*., 2003; Ahemad and Khan, 2011a).

In similar studies, the recommended rate of fungicide for example captan reduced nodulation and N_2 fixation by *Trifolium repens* (Fisher and Hayes, 1981). Fungicides like, thiram and captan have also been reported to be harmful to nodulation and N_2 fixation of several grain and forage legumes (Heinonen-Taski *et al.*, 1982; Rennie *et al.*, 1985; Aamil *et al.*, 2004).

CONCLUSIONS

In conclusion, a varying degree of toxicity of tebuconazole was observed to the tested legumes. However, tebuconazole displayed the maximum toxicity to shoot growth, leghaemoglobin, chlorophyll, shoot N, shoot P and seed protein in chickpea; root growth and nodule numbers in pea; root and shoot P in greengram; nodule biomass, root N and seed yield in lentil. Generally, tebuconazole showed the most adverse effect on the growth parameters of chickpea and affected the least the greengram growth parameters.

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