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EFFECT OF FUNGICIDES ON NITRATE UPTAKE AND ASSIMILATION IN *ASPERGILLUSNIDULANS*

U. Amin and *N. Akhtar

Institute of Agricultural Sciences, University of the Punjab, Lahore
Corresponding Author E-mail: naureenshahrukh@yahoo.com

ABSTRACT

In present study, effect of three fungicides, Iprobenfos, Fosetylaluminium and Azoxystrobin, on substrate specificity of NrtA and NrtB nitrate transporter proteins of *Aspergillus nidulans* has been studied. Azoxystrobin inhibited the growth of *A. nidulans* strains more strongly than Iprobenfos and Fosetyl aluminium. At0.25R of Iprobenfos, approximately 82%, 85%, 76% growth inhibition was observed by wild type, *nrtA1* and *nrtB110*respectively. Growth inhibition of wild type, *nrtA1* and *nrtB110* by recommended dose (R) of Fosetyl aluminium was 73%, 41% and 23%, respectively. 0.0075R of Azoxystrobin inhibited the growth of wild type, *nrtA1* and *nrtB110*by 51%, 68% and 71%, respectively. Effect of Iprobenfos, Fosety laluminium and Azoxystrobinwas also studied on nitrate uptake of the *A. nidulans* strains. At 0.25R of Iprobenfos, 94%, 54% and 100% nitrate uptake inhibition was observed by wild type, *nrtA1* and *nrtB110* respectively. Recommended dose of Fosety laluminiumcompletely stopped the nitrate uptake by all strains. At 0.01R of Azoxystrobin, complete cessation in nitrate uptakeby *nrtA1* and *nrtB110*was recorded however78% reduction in nitrate uptake was observedfor wild type strain.Increase in concentration of Iprobenfos(up to 0.25R), increasedthe nitrate reductase activity in wild type while decreased the enzymatic activity by*nrtA1* and *nrtB110*strains. Recommended dose of Fosetylaluminiumand 0.0075R of Azoxystrobinenhanced nitrate reductase activity in wild type and *nrtB110*, however reduced theenzymatic activity in *nrtA1*. Present study concludes that excessive use of fungicides is not only a threat for environment but also reduces the nitrate uptake and assimilation efficiency of eukaryotes.

Key words: *Aspergillus nidulans,*growth inhibition, nitrate uptake, pesticides.

INTRODUCTION

Nitrogen is the fifth most abundant element in nature and is an important macro nutrient that is crucial for biological molecules such as nucleic acids and amino acids, hence important for plant growth. Nitrate is the chief source of nitrogen in soils that not only stimulates organism's growth by inducing genes that encode nitrate transporter proteins or enzymes taking part in nitrate assimilation but also positively affects plant morphogenic processes (Scheible *et al.,* 1997a), root development (Zhang and Forde, 1998) and carbon metabolism (Scheible *et al.,* 1997b). Negatively charged nitrate molecules cannot enter rapidly in cell membrane because of the membrane potential and concentration gradient. That is why entry of the nitrate into the cells is accomplished by active transport systems (Clegg *et al.,* 2002). Nitrate concentration fluctuates widely depending upon regional and seasonal variations. To cope up with this nitrate fluctuation, plant roots have developed two uptake systems. One of the systems is low affinity transport system and other is high affinity transport system (Orsel *et al.,* 2002).For last six decades, *Aspergillusnidulans*has been studied broadly to understand the process of nitrate transport and metabolism that enabled us to comprehend this pathway. Nitrate assimilation in *A. nidulans* is genetically and biochemically same as in higher plants so the information can be related to plants (Unkles *et al.,* 2004).Nitrate transport in *A. nidulans* is accomplished by two high affinity nitrate transporters NrtA and NrtB, encoded by *nrtA* (previously designed *crnA*) and *nrtB* genes, respectively (Brownlee and Arst, 1983; Unkles*et al.,* 2001).

Besides the benefits of pesticides, their wide and continuous use has produced several problems concerning environment pollution. Pesticides are badly contaminating the environment because of their complex degrading property by offsite mobilization to ground and surface water, effecting non target organisms (Hafez and Theimann, 2003), affecting soil organisms that leads to poor soil fertility (Schuster and Schröder, 1990) and negatively influencing the human health by their bio-accumulation in food chains. Recently, a sturdy increase in amount of pesticide residues has been noticed in our soil and food (Ubuoh *et al.,* 2012). Non target organisms are endangered by accumulation of these biologically active residues (Mishra, 2001).

Present study was designed to study the toxicity levels of fungicides on *A. nidulans* strains as well as to determine the degree of toxicity of fungicides on nitrate transporter proteins and nitrate reductase enzymes in *A. nidulans*.

MATERIALS AND METHODS

Selection of strains: The impact of commercially available different fungicides on growth, net nitrate transport and nitrate reductase activity of *A. nidulans* mutant strains was studied. The selected mutants of *A. nidulans* were different from each other with respect to the presence or absence of one or both of the nitrate transporter proteins, NrtA and NrtB (Table 1).

Table 1.*A. nidulans* mutant strains used in present study.

*A. nidulans*strains	Functional transporter(s)	Reference/origin
Wild type	NrtA, NrtB	Department of Genetics, University of Glasgow, UK
nrtA1	NrtB	Arst and Cove, 1973
nrtB110	NrtA	Unkles*et al.*, 2001
T110	None	Unkles*et al.*, 2001

All the four mutant strains were obtained from Fungal Genetics Laboratory, School of Biology, University of St Andrews, UK.All these strains were grown and maintained on complete or minimal *Aspergillus* medium as described by Clutterbuck (1974).

Strain T110 was mutant in both transporter genes so was unable to grow on medium having nitrate as sole source of nitrogen. Therefore was not studied in future experiments.

Selection of pesticides: Commercially available potential systemic fungicides; namely Azoxystrobin, Fosetylaluminium and Iprobenfos (Akhtar *et al*., 2015b)were evaluated for their efficacy to inhibit the growth of selected mutant strains. All the selected fungicides control pathogens by altering or inhibiting their metabolic pathways. Fungicide solutions were prepared by making their stocks in water and then required amount of this pesticide solution (calculated according to their recommended dose) was added to the minimal medium. The fungicide solutions were prepared fresh, when required. Chemical constituents and recommended doses of the fungicides used for present study are as follows:

Table 2. Detail of fungicides used in present study.

Fungicide	Recommended dose (R) /100L H_2O
Azoxystrobin	180 ml
Fosetylaluminium	250 g
Iprobenfos	200 ml

Growth inhibition studies: Growth inhibition tests by fungicides were performed on solid minimal medium supplemented with 1mM $NaNO_3$ as sole nitrogen source. Fungicide amended medium was inoculated with 10 spores of individual strain (5µl of spore suspension having $2x10^3$ spores/ml).Each treatment was made as replicate of four and all strains were inoculated in one petriplate to minimize the experimental error. Each treatment was made in triplicates. Control treatments were made by inoculating the strains in the same way on growth medium without fungicide. All inoculated petriplates were incubated at 25º C ±2 for 3 days in a completely randomized design and scored for their growth.

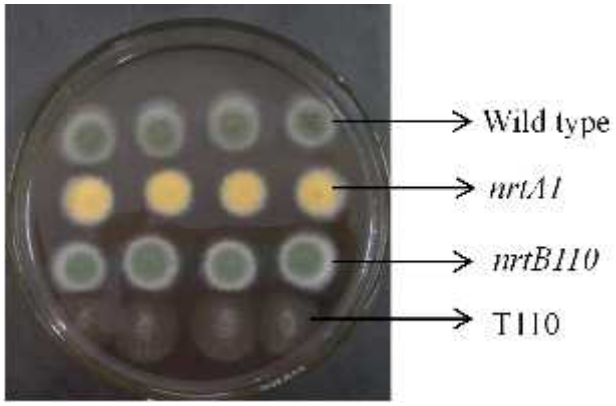

Fig. 1: Pattern of strain inoculation in petriplates. Strain T110 showed very poor growth on nitrate amended medium. Although all strains are of *A. nidulans* but showed variable colony color due to altered metabolic pathways by induced mutation(s)

Inhibition of fungal biomass production: Biomass inhibition studies were carried out on broth minimal medium supplemented with 1 mM nitrate. Freshly prepared fungicide solutions were added to the minimal medium as required and inoculated with 10^5 spores. Control treatments were made without fungicides for comparison. Each treatment was triplicated. These flasks were incubated at 37 ºC at 200 rpm. After 3 days of incubation, fungal mycelia were collected by filtration on pre-weighed filter papers and oven dried overnight at 50 ºC to record the dry biomass (mg). Percentage inhibition in fungal fresh and dry biomass was calculated using the formula

$$\% \text{ inhibition} = \frac{\text{Biomass in control (mg)} - \text{Biomass in treatment (mg)}}{\text{Biomass in control (mg)}} \times 100$$

Effect of pesticides on net nitrate uptake: Effect of pesticides on net nitrate uptake was determined by measuring the depletion of nitrate in the assay medium (Brownlee and Arst, 1983; Akhtar *et al.*, 2015a). Conidia were harvested from the whole petriplate of pure culture of strain to make the spore suspension in 10 ml sterilized

saline Tween 80 (0.9% (w/v) NaCl, 0.1% (v/v) Tween 80) and then vortexed thoroughly. 200ml minimal medium supplemented with 5mM urea, as sole source of nitrogen, was inoculated with this spore suspension and incubated at 37ºC for 16hr with constant shaking at 200rpm. 10mM $NaNO_3$ was added to induce the transporter protein(s) 100 min prior to harvest. Standard curve for nitrate was prepared by taking OD of the mixture 50µl of nitrate concentration (0, 100, 200, 300, 400, 500µM) and 950µl 5% perchloric acid at 204nm.

For net nitrate uptake assays, 50ml minimal growth medium was poured in 250ml conical flask. This medium was supplemented with 500µM $NaNO_3$ and required amount of pesticide. For each treatment, a 50ml of aliquot of growth medium having fungal cells was rapidly filtered under vacuum on Millipore membrane. These cells were oven dried at 60 ºC for 24hr to determine the fungal dry biomass.

50ml cells were filtered through a nitrogen free Millipore membrane filters using vacuum pump, washed 3-4 times with 50ml pre-warmed distilled. Membrane filter along with mycelium was transferred into the pre-warmed assay medium supplemented with pesticide. Quickly, 3ml medium with cells was filtered rapidly under vacuum and used as a 0 time reading. Assay flask was incubated at 37 ºC, 200rpm for 20 min and aliquot of 3ml was taken again filtered rapidly to serve as 20 min reading.

To measure rate of net nitrate uptake, 50µl filtrate and 950µl of 5% perchloric acid were mixed and absorbance was taken at 204nm by UV visible spectrophotometer. Net nitrate uptake was determined as nmole nitrate taken per mg fungal dry weight per min.

For net nitrate reductase activity, 50ml aliquot of minimal medium was filtered through miracloth, washed with cold distilled water and press dried. This mycelium was ground with liquid nitrogen three times and added to 3ml 0.1M sodium phosphate buffer (pH 7.0) with gentle shaking at 40rpm for 30 min. This suspension was centrifuged at 15000rpm for 20 min at 4ºC. Supernatant was collected and placed on ice. These fungal extracts were diluted 1/20 in 0.1M sodium phosphate buffer. Another 50ml aliquot of cells was filtered for dry fungal biomass and oven dried at 60 ºC for 24hrs.

To measure the rate of nitrate reductase, a mixture of 160µl of 50mM sodium phosphate buffer (pH, 7.75), 8µl of 1M $NaNO_3$, 2µl of 1M $NaSO_3$, 40µl of 1mM FAD, 30µl sterilized cold distilled water, 80µl diluted fungal extracts and required amount of fungicide was mixed. Reaction was started by adding 80µl of 1mM NADPH and reaction mixture was incubated at 25 ºC for 20 min. 400µl 1% sulfanilamide (prepared in 25% HCl) and 400µl 0.2% NED were added and OD at 540nm was recorded using UV visible spectrophotometer after 10 min. nitrate reductase activity was determined in pmol/min/mg dry weight.

RESULTS

The experiments were performed to examine the impact of fungicides on the activity of nitrate transporter proteins, NrtA and NrtB, of *Aspergillus nidulans* encoded by *nrtA* and *nrtB* genes respectively. Three different pesticides namely Azoxystrobin, Fosetyl aluminium and Iprobenfos were selected to study their effect on growth, net nitrate uptake and nitrate reductase activity of *A. nidulans* using wild type and mutant strains in one transporter or the other.

Fungal growth sensitivity towards Iprobenfos: Growth sensitivity of wild type and mutant strains towards Iprobenfos was assessed by growing the fungal strains in medium amended with 0 to 0.25R of this pesticide and 1mM sodium nitrate as sole source of nitrogen. All the four strains demonstrated the same decreasing growth pattern as the concentration of Iprobenfos increased in growth medium. No growth was observed in wild type, *nrtA1* and *nrtB110* above 0.25R of Iprobenfos(Fig2).

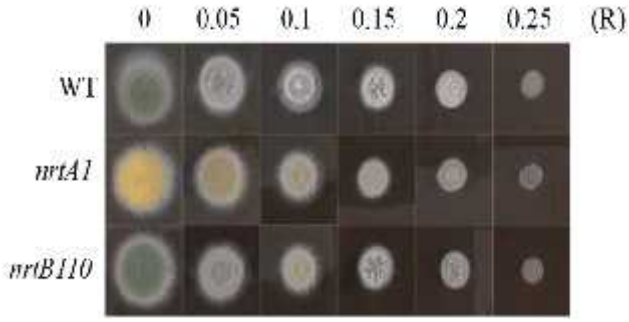

Fig 2: Growth sensitivity of strains to Iprobenfos. Strains were grown in fungicide amended medium for 3 days in 9mm petriplate.

Concentrations of Iprobenfos used to detect the reduction in fungal biomass production were 0, 0.1, 0.15, 0.2 and 0.25R. Results indicated that dry weight of the fungus decreased as the concentration of pesticide increased (Fig 3). At 0.25R of Iprobenfos, approximately 82% inhibition in wild type growth, 85% inhibition in case of *nrtA1* mutant and 76% reduction in *nrtB110* growth was recorded.

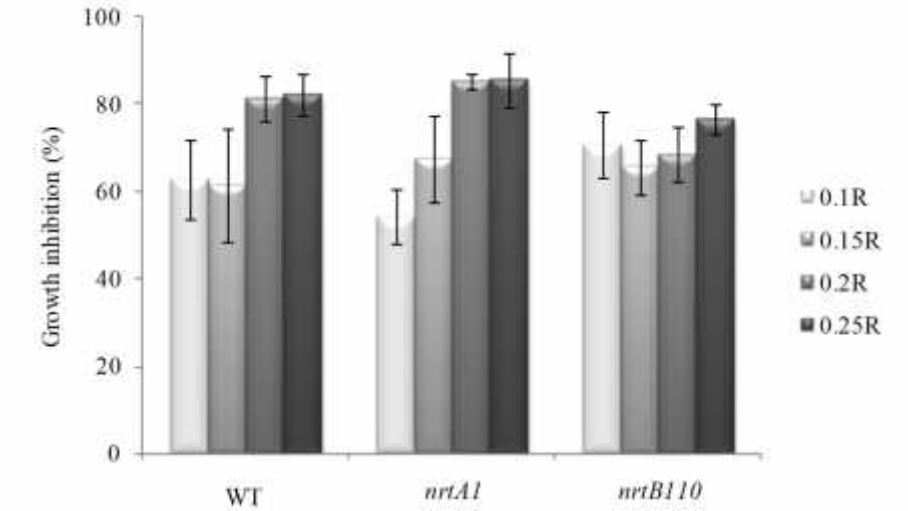

Fig 3: Effect of Iprobenfoson biomass production of *A. nidulans*strains.

Fungal growth sensitivity towards Fosetylaluminium: For petriplategrowth assays, different concentrations up to recommended dose (R) of Fosetylaluminium were tested. Similar inhibition in growth was observed for wild type and both mutant strains. A gradual decline in fungal colony diameter was recorded by increasing the fungicide concentration from 0.1R to 0.75R and complete inhibition at recommended dose (R) of Fosetylaluminium (Fig 4).

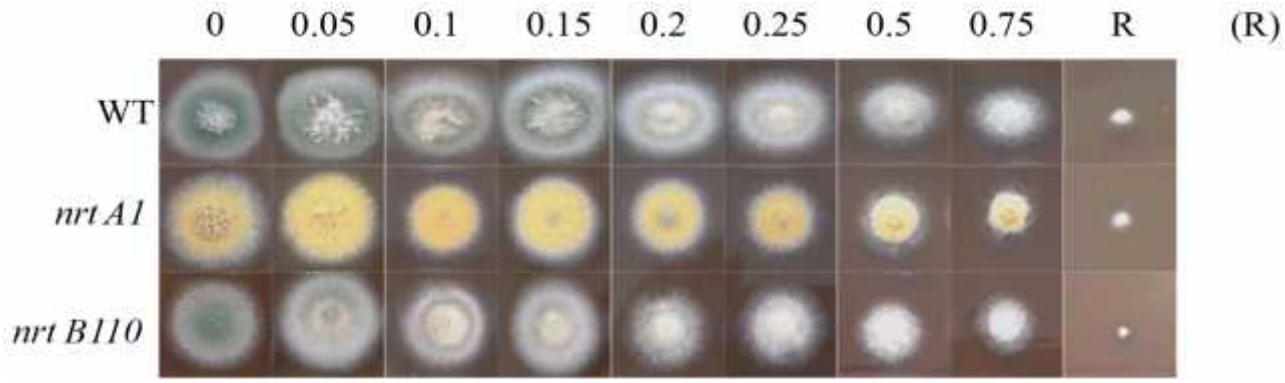

Fig 4: Growth sensitivity of strains to Fosetylaluminium. . Strains were grown in fungicide amended medium for 3 days in 9mm petriplate.

Different concentrations of the Fosetylaluminium (0, 0.25, 0.5, 0.75 and R) were evaluated for their potential to inhibit the biomass production of strains (Figure 5). On 1mM $NaNO_3$, growth of the wild type strain inhibited up to 73% at R while*nrtA1* mutant strain exhibited 41% reduction in growth at R of Fosetylaluminium. However R of Fosetylaluminium could inhibit about 23% growth of mutant strain *nrtB110.*

Fungal growth sensitivity towards Azoxystrobin: Almost similar growth responses were observed for wild type and mutant strain *nrtB110* when grown on medium amended with 1mM $NaNO_3$ and Azoxystrobin. Growth of the both strains was restricted by 0.25R of the Azoxystrobin while for mutant *nrtA1*, 0.05R of the Azoxystrobinwas sufficient to inhibit the growth on 1mM nitrate (Fig 6).

Results of *A. nidulans* dry biomass production indicated that a very little dose of Azoxystrobin can negatively affect the growth of strains. Five concentrations 0, 0.0025, 0.005, 0.0075 and 0.01 of recommended dose (R) of Azoxystrobin were applied on the strains. Growth inhibition of the wild type strain was up to 51% at 0.0075R, *nrtA1* demonstrated growth inhibition upto 68% at 0.0075R with a little increase in growth at 0.01R of Azoxystrobin. Azoxystrobin resulted in growth inhibition up to 70% at 0.01R (Fig 7).

Net nitrate uptake assays: Nitrate uptake assays were conducted for wild type and mutant strains, *nrtA1* and *nrtB110*, possessing nitrate transporter protein/s (NrtA,

NrtB). These assays were carried out with the pesticides (Iprobenfos, Fosetylaluminium and Azoxystrobin) that exhibited the negative impacts on the growth and biomass production of *A. nidulans* strains.

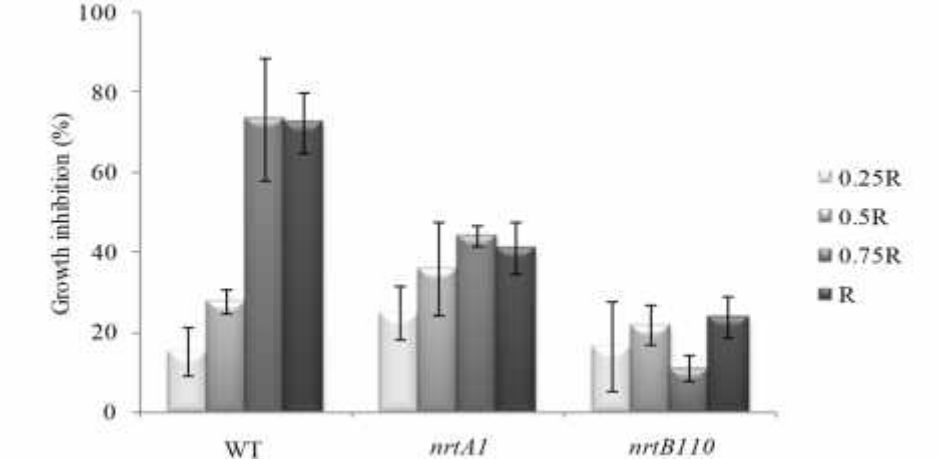

Fig 5: Effect of Fosetylaluminiumon biomass production of *A. nidulans*strains.

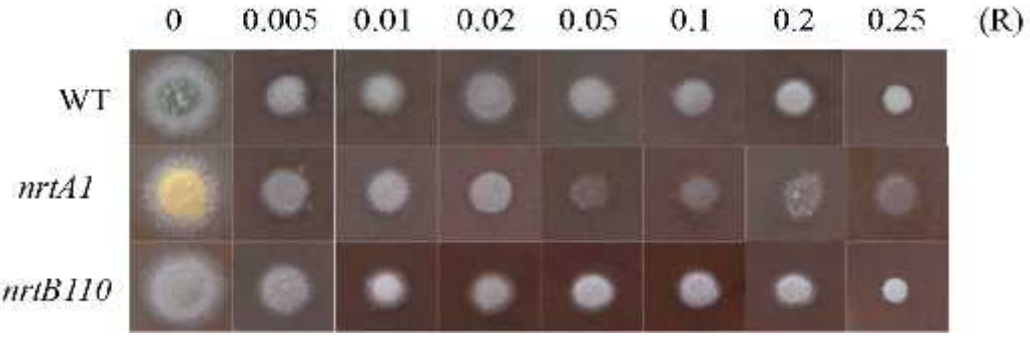

Fig 6: Growth sensitivity of strains to Azoxystrobinin the presence of nitrate. Strains were grown in fungicide amended medium for 3 days in 9mm petriplate.

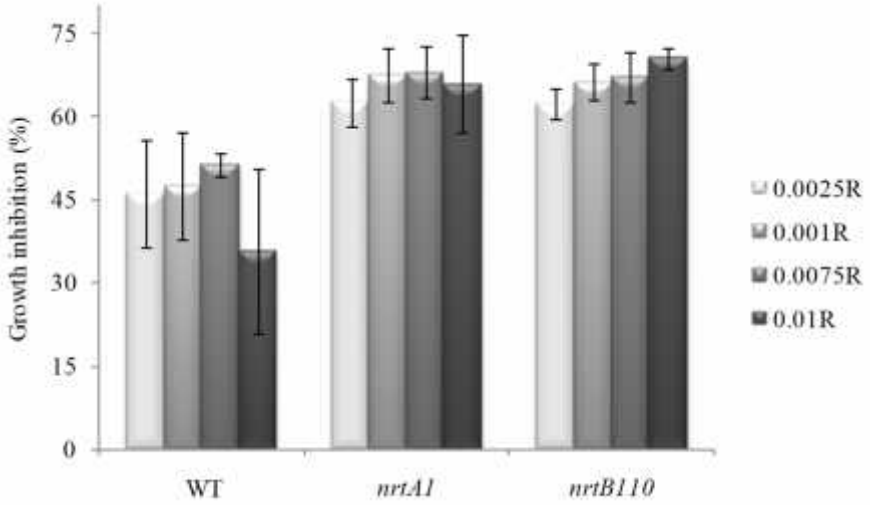

Fig 7: Effect of Azoxystrobinon % growth inhibition of *A. nidulans*strains.

Increasing concentration of Iprobenfos equally affected the net nitrate uptake by wild type and *nrtB110* whereas nitrate uptake by *nrtA1* was severely affected as compared to other strains. A dose of 0.25R Iprobenfos completely inhibited the nitrate uptake by *nrtB110* while, 0.25R of Iprobenfos reduced the nitrate uptake rate

upto94% in wild type strain that was 0.84nmol/min/mg dry weight as compared to its control, 13.11nmol/min/mg dry weight. Other doses of Iprobenfos tested, 0.1 R, 0.15 R, 0.2R inhibited the nitrate uptake of wild type strain up to 9%, 13%, 73%, respectively. Whereas, approximately 14%, 20% and90% decreased in nitrate uptake by *nrtB110* was observed by 0.1R, 0.15R, and 0.2R of Iprobenfos, respectively. Inhibition pattern in *nrtA1* was also concentration dependant that exhibited 18%, 38% and 47% by 0.1R, 0.15R and 0.25R of Iprobenfos respectively followed by maximum inhibition (54%) at 0.25 R of Iprobenfos with nitrate uptake rate of 1.53nmol/min/mg dry weight as compared to its control, 3.63nmol/min/mg dry weight (Fig8).

Inhibition of nitrate uptake at 0.25R and 0.5R of Fosetylaluminium by wild type was 39% and 50%, by *nrtA1* was 43%, 56% and by *nrtB110*it was 40%, 52%, respectively. 0.75R of Fosetylaluminium was enough to inhibit nitrate uptake up to 94%, 72%, 69% by wild type, *nrtA1* and *ntrB110* respectively that was observed by reduction in nitrate uptake to 0.55, 0.97, 2.6nmol/min/mg dry weight respectively. Rate of nitrate uptake decreased as the concentration of fungicide increased, at the recommended dose (R) of Fosetylaluminium the uptake ability of the all strains or both transporters transporter was completely lost (Fig9).

Results of increasing concentration of Azoxystrobinon *A. nidulans* strains indicated that mutant strains, *nrtA1* and *nrtB110*, were adversely affected by Azoxystrobin than wild type strain. *nrtA1* and *nrtB110* exhibited 100% nitrate uptake inhibition at 0.01R of Azoxystrobin while at the same concentration, nitrate uptake by wild type was inhibited up to 78%. Nitrate uptake by *nrtA1* and *nrtB110* at 0.0025Rwere 3.85 and 7.11nmol/min/mg dry weight, at 0.005R was 2.55 and 4.05nmol/min/mg dry weight, at 0.0075R was 1.4 and 3.94nmol/min/mg dry weight as compared with the control values i.e3.78nmol/min/mg dry weight and 8.88nmol/min/mg dry weight, respectively. Concentration dependant inhibition pattern of wild type strain demonstrated the final reduction in nitrate uptake to 0.55nmol/min/mg dry weight at 0.01R of Azoxystrobin. For other concentrations tried, nitrate uptake inhibition by wild type was 43%, 54%, 62% at 0.0025R, 0.005R, 0.0075R of Azoxystrobin, respectively (Fig10).

Nitrate reductase activity assays: Different concentrations of Iprobenfos, Fosetylaluminiumand Azoxystrobin were used to examine their effect on nitrate reductase rate (conversion of NO^{-}_{3} to NO_2) by *A. nidulans* strains (wild type, *nrtA1* and *nrtB110*). Nitrate reductase rate of wild type strain increased to 6.56pmol/min/mg dry weight at 0.25R of Iprobenfos while that of *nrtA1* and *nrtB110* decreased to 5.80pmol/min/mg dry weight and 2.30pmol/min/mg dry weight, respectively, with a non significant difference at 0.25R of Iprobenfos (Table 3).

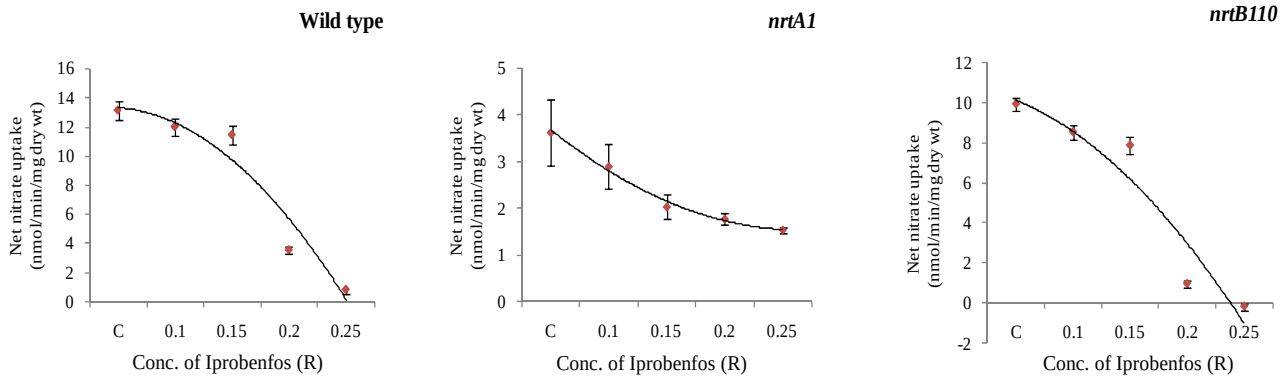

Fig8: Effect of Iprobenfoson net nitrate uptake by*A. nidulans*strains.

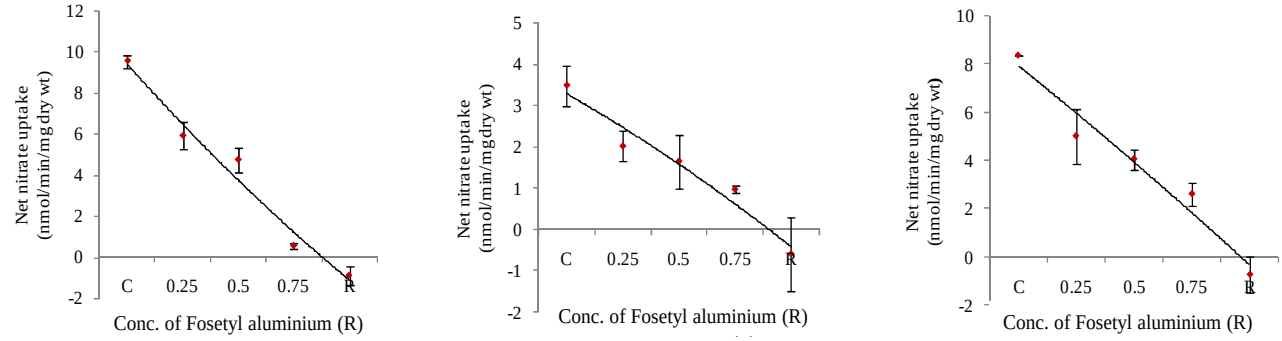

Fig9: Effect of Fosetylaluminiumon net nitrate uptake by*A. nidulans*strains.

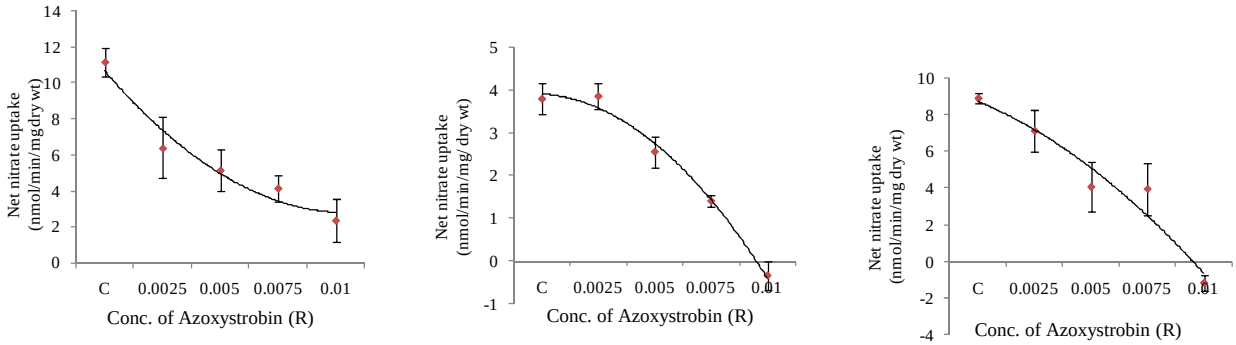

Fig10: Effect of Azoxystrobinon net nitrate uptake by*A. nidulans*strains.

Table 3.Fungicide effect on nitrate reductase activity of *A. nidulans* strains.

Fungicide	Conc. of fungicide	Nitrate reductase rate (pmol/min/mg dry weight)		
		WT	***nrtA1***	***nrtB110***
No	Control	4.93 ± 0.38	6.01 ± 0.36	2.77 ± 0.03
Iprobenfos	0.1R	6.49 ± 0.04	5.76 ± 0.51	2.32 ± 0.08
	0.25R	6.56 ± 0.16	5.80 ± 0.60	2.30 ± 0.07
Fosetylaluminium	0.5R	5.94 ± 0.25	5.29 ± 0.32	3.13 ± 0.20
	R	6.73 ± 0.33	5.40 ± 0.38	3.24 ± 0.28
Azoxystrobin	0.0025R	5.47 ± 0.22	5.07 ± 0.56	2.83 ± 0.36
	0.0075R	5.36 ± 0.07	4.96 ± 0.32	2.86 ± 0.09

Results indicated that wild type and *nrtB110* demonstrated enhanced nitrate reductase rate of 6.73 and 3.24pmol/min/mg dry weight at R of Fosetylaluminium. On the other hand, *nrtA1* exhibited non significant reduction in nitrate reductase rate to 5.4pmol/min/mg dry weight at R of Fosetylaluminium (Table 3).Effect of increasing concentration of Azoxystrobinon *A. nidulans* strains indicated that 0.0075R of Azoxystrobin decreased the nitrate reductase rate of *nrtA1* from 6.01 to 4.96pmol/min/mg dry weight while increase in nitrate reductase rate to 5.36 and 2.86pmol/min/mg dry weight was noted down in wild type and *nrtB110* from their control values 4.93 and 2.77pmol/min/mg dry weight, respectively, at the same concentration (Table 3).

DISCUSSION

The current investigation was conducted to identify the possible ways of minimizing negative impacts of widely used pesticides, affecting agriculture and human environment. This study revealed enormous harms of pesticides on the performance of nitrate transporter protein genes and nitrate reductase of the tested *A. nidulans*strains.

Pesticides are well known for their beneficial effects in crop productivity and pest control but they are also toxic to non target organisms (Diez, 2010). There is need of wise selection and application of pesticides at their minimum effective dosage. Pesticides affect soil microorganisms by interfering their metabolic behavior (Singh and Walker, 2006), modify their biochemical and physiological activities (Hussain *et al.*, 2009).

In vitro studies are used to determine inhibition mechanisms and toxicity of particular pesticides (Locke and Zablotowicz, 2004). Three pesticides Azoxystrobin, Fosetylaluminium and Iprobenfos were tested for nitrate uptake and nitrate reductase assays due to their inhibitory effects on growth of *A. nidualns* strains during petriplate experiments. Fungal biomass production assays were continued keeping in view the minimum inhibition concentration of pesticides. Microbial biomass, a significant indicator of microbial behavior, provides evaluation of association between nutrient conversions and other ecological processes (Schultz and Urban, 2008). Several current studies have disclosed the negative effects of pesticides on soil microbial biomass (Pampulha and Oliveira, 2006). Complete inhibition of *A. nidulans* growth has been already been reported by recommended dose of Fosetylaluminium, Iprobenfosand Azoxystrobin (Akhtar *et al.*, 2015b). In present study, 0.25R of Azoxystrobin and Iprobenfosand R of Fosetylaluminium were enough to inhibit the growth, revealing the fact that NrtA and NrtB transporter proteins are very sensitive to Iprobenfosand Azoxystrobin. However, Fosetylaluminium equally affected the wild type and other mutant strains. This may be due to the selective permeability of cell membranes for these fungicides (Yang *et al.*, 2011).

Fosetyl aluminium has also been reported for its antifungal activity against various biotrophs and nectrotrophs (Cohen *et al*., 1987). A significant

antifungal activity of Iprobenfosis because of its active ingredient, Iprobenfos that is reported to inhibit phopholipids synthesis as an action against fungi (Roberts and Hutson, 1999).

0.25R of Iprobenfos inhibited 100% nitrate uptake by *nrtB110*, 54% by *nrtA1* and 93% by wild type indicating complete inhibition of NrtA protein. 100% inhibition of wild type, *nrtA1* and *nrtB110* at R of Fosetylaluminium exhibited complete inhibition of nitrate uptake by NrtA and NrtB transporter proteins. 0.01R of Azoxystrobin inhibited 100% nitrate uptake by *nrtA1* and *nrtB110* and 78% by wild type. Results with mutant strains showed that 0.01R of Azoxystrobin completely inhibited nitrate uptake by NrtA and NrtB transporter proteins while some nitrate uptake was observed by wild type strain. Akhtar and colleagues (2015a) reported the differential behaviors of both transporters as cesium is the inhibitor of NrtBonly and sulphite can inhibit the activity of both NrtA and NrtB.

Enzyme activities are affected by soil conditions and pesticide application rate (Locke *et al.,* 2004). Metabolic or enzymatic activities can be disturbed by pesticides (Engelen *et al.,* 1998; Liu *et al.,* 2008). Several workers have reported adverse effects of pesticides on activities of soil enzymes i.e. dehydrogenase, hydrolases and oxidoreductases (Malkomes, 1989; Perucci and Scarponi, 1994; Ismail *et al.,* 1998; Monkiedje and Spiteller, 2002; Menon *et al.,* 2005). Present study revealed the effects of Iprobenfos, Fosetylaluminium and Azoxystrobinon nitrate reductase activity of *A. nidualns* strains. With increasing concentration of Iprobenfos(up to 0.25R), increase in nitrate reductase activity was observed for wild type while activity was decreased for *nrtA1* (although a non-significant difference) and *nrtB110*. Malkomes (1997) described such differences to the dual behavior of pesticides (both positive and negative). Various factors including pesticides chemical nature and concentration used can contribute to deviating research conclusions. R of Fosetylaluminium and 0.0075R of Azoxystrobin accelerated the nitrate reductase activity of wild type and *nrtB110* with reduced activity in *nrtA1* in present study. Increase in soil enzymatic activities and ATP content is evident by application of some pesticides (Shukla, 1997; Megharaj *et al.,* 1999). Increase, decrease or neutral effect due to pesticide application of enzymes like nitrate reductase depends upon nature and concentrations of pesticides used, incubation period, status of enzymes in soil. Restricted nitrate flux stimulated by fungicide stress can lead to decrease in nitrate reductase activity because of enzyme degradation or inactivation (Ferrario *et al*., 1998).Abdel-Mallek and colleagues (1994) has reported inhibition of nitrate reductase activity by Profenofos.

Conclusion: The present study is concluded that the continuous, excessive and misuse of pesticides has potential hazards for soil mycoflora as well as for nitrate assimilation and transport by soil organism growing in the pesticide affected soil.

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