

Original Research Article

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Impact of Propiconazole on Soil Enzymes Activity in Tropical Red and Paddy Soils

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Abstract

The use of triazole fungicide as agrochemicals into soil leading to many long lasting effects on the microbes present in the soil environment which indirectly affecting the soil health, the current work evaluates the impact of propiconazole on cellulase and invertase activities in red loam soil of groundnut (*Arachis hypogaea* L.) and paddy soil (*Oryza sativa*). *In vitro* experiment was conducted to evaluate the effect of propiconazole on their application rates (1.0, 2.5, 5.0, 7.5 and 10.0 kg/ha) on cellulase and invertase. The period of experimental set up was 10, 20, 30 and 40 days under the *in vitro* conditions. The results show that propiconazole was found stimulatory for the activities to the cellulase and invertase at lower application rates in both soils after 10 days of incubation period and an outstanding increase in the activity of cellulase and invertase was observed at the application rate 5.0 and 2.5 kg/ha respectively. Conversely, the comparable trend was observed after 20 days of incubation. The higher application rates (7.5 and 10.0 kg/ha) were found toxic to activity of cellulase and invertase in both red loamy and paddy soil. However, the results of the present investigation suggest that, recommended application rates of propiconazole have greater impact on the enzyme activity in the soil and the application of higher doses and regular contact of propiconazole will cause the harmful effect on enzyme activity in red loamy and paddy soil.

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Triazole fungicide

Introduction

Agricultural production in the Republic of India magnified dramatically throughout the last four decades, resulting in an era of food self-reliance and this can play a key role in the growth of Indian economy. Significantly, this growth signifies the assembly of 28 share of the gross domestic product (GDP) and up to date exceptional growth was achieved through the uptake of newer technologies within the sort of high yielding

crop varieties, chemical fertilizers and pesticides. It's additionally vital to understand that Indian agriculture has advanced protracted manner from an amount of frequent droughts and susceptibility to food scarcities (Abrol and Sangar, 2000).

Enzymes are the active agents for life courses, similarly, in the soil they are recognized to show a considerable part in preserving the soil healthiness and its environment. The activity of enzyme in the

soil is generally of microbial origin, being resulting from cell-associated or free enzymes and intracellular. Meanwhile, a soil health is maintained with equal contribution of physical, chemical and biological components (Ellert et al., 1997). Cellulase is a one of the important enzyme in the carbon cycle and it specifically acts on the β -1,4-glucan bonds in cellulose, and it is a rich carbonaceous polymer in the environment, Therefore, cellulase involved in the main routes of the carbon cycle (White 1982; Mallik and Sharma, 2002). Apparently, Invertase catalyses the hydrolysis of sucrose to fructose and glucose, moreover and is extensively dispersed in microorganisms, plants and animals (Alef and Nannipieri, 1995).

The toxicant impact of the many fungicides is still unknown, Shukla (2000) has demonstrated the toxicant impact of fungicides on microbial population and impact on enzyme activity, it's clear that once the antimycotic agents are in contact with soil can end up in the modification within the activity of soil enzymes. As a result, the considerably soil catalyst activity is down (Shetty and Magu, 1998). Triazole area unit, the key cluster of fungicides they're used wide owing to their versatile nature and general action. In spite of their wide unfold usage, the toxicity analysis weren't comprehensively assessed (Filipov and Lawrence, 2001).

Wightwick et al. (2010) reports regarding environmental risks of triazole fungicides on agriculture production systems, it provides clear clues that triazole contaminations will result in the dramatic decrease within the production of the agricultural merchandise. Propiconazole could be a triazole foliar antimycotic agent, employed in the agriculture fields. The yearly intake of this antimycotic agent is regarding 7373 g ai./ha. It's one amongst the leading triazole antimycotic agent within the agriculture field in the India. Propiconazole is used to protect the crops of wheat, rice, tea, banana and etc. against the diseases like karnal bunt, brown rust, black rust, yellow rust, sheath blight, blister blight and etc. The toxicity assessment isn't clearly studied. However, antecedent few researchers studied the impact of propiconazole on the soil microbial population and its impact on crop yield (Elmholt, 1991; Elmholt, 1992; Yen et al., 2009). Hence, the current investigation was undertaken to check the impact of propiconazole antimycotic agent on soil enzyme activity.

Materials and methods

Soil used for the enzymes activity studies

To know the impact of propiconazole on soil enzyme activity, two different soils were chosen for this study, red sandy loamy soil from the ground nut cultivated field and paddy soil from the Dharwad district, Karnataka, India, it was collected from the upper layer (0-10 cm) wherever the microorganism population is rich with high enzyme activity. Both the soil was aseptically brought to the laboratory in sterile baggage. Soils were mixed completely for the preparation of homogeneous composite mixture; all the debris and plant materials were removed. Further, the soil samples were sieved through 2 mm sieve and dried at 30°C in hot air oven (JSR). Air dried soil samples were stored in the 4°C before analysis.

Fungicide used for the soil enzymes activity

To evaluate the impact of propiconazole on the soil enzyme activities, Tilt 25 EC (propiconazole antimycotic 25th blended grade, Syngenta, India) was procured from native pesticide dealers of Dharwad. The emulsified grade of propiconazole fungicide dissolved within the distilled water 25ml: 100L ratio for the further use.

Physicochemical properties of soils

The content of sand, silt and clay as mineral matter within the red loamy was evaluated by the subsequent method of Alexander (1977). The amount of nitrogen content within the soil was analyzed by Micro-Kjeldhal methodology (Jackson, 1971). The percentage of water holding capability of the soil throughout the experiment was calculated according to the Johnson and Ulrich (1960). Using the digital pH scale meter, the pH of soil was determined by the quantitative relation of 1:1.25 of soil to water.

Soil samples preparation

A homogenate composite mixture of soil samples was weighed specifically 5 grams and dispersed into the clean sterile test tube (25 × 150 mm). Antecedently prepared stock solutions of commercial grade propiconazole antimycotic was added at the rate of 10, 25, 50, 75 and 100 µg/g, which are equal to the rate of field application i.e. 1, 2.5, 5, 7.5 and 10 kg/ha,

respectively. Antimycotic content tube was considered as the positive tubes, whereas, the tubes only with soil however not antimycotic were treated as the control tubes. All the samples were mixed completely for the higher circulation of fungicide. For all the treatments, three sets of experiments were maintained throughout the 40 days of study at the $28 \pm 2^\circ\text{C}$ in a humid atmosphere (75-80% relative humidity).

Cellulase activity (EC 3.2.1.4.)

With the intention of determining the cellulase enzyme activity of the soils, at the preferred intervals, the method described by the Srinivasulu and Rangaswamy (2006) was adopted for the analysis. All the samples were extracted using distilled water for the analysis of enzyme activity. The extracts were transferred to the Erlenmeyer flasks and to prevent the enzyme activity the inhibitor was added and incubated for the 30 minutes, moreover 1% of 10 ml carboxy methyl cellulose was used as the substrate followed by the 10 ml of acetate buffer were added and incubated for the 24 hrs to know the reducing sugar concentrations within the extracted samples (Deng and Tabatabai, 1994). In the other hand, the activity of cellulase was evaluated at 10, 20, 30 and 40 days of incubation at $28 \pm 2^\circ\text{C}$ with higher than mentioned particular enzyme substrate.

Invertase activity (EC 3.2.1.26)

The operating method for the assay of invertase was developed by the Cole (1977) and followed by the Tu (1981). The fractions of soil samples were transferred to the Erlenmeyer flasks and 1 ml of resolvent was

additional to flasks to arrest the enzyme activities, after the desired 15 minutes of incubation time. A 18 mM of 6ml sucrose was added to the actual soil samples and incubated for the 24 and 28 hrs, the test samples were filtered by the Whatman filter paper no. 1 and obtained filtrates were analyzed for the assessing the concentration of glucose present in the soil samples by the Nelson Somogyi methodology (Nelson, 1944) in Hitachi U2900 spectrophotometer. In the other side, the enzyme activity of the invertase was assayed at 10, 20, 30 and 40 days of the time period with appropriate enzyme substrate.

Statistical analysis

All the experiments were carried in a triplicates and the rate of concentrations of cellulase and the amylase deliberate based on the soil weight. Obtained information was assessed by using one way ANOVA with the Post hoc DUNCAN'S test by using the SPSS 20 statistical data software package, all the data were analyzed at $p \leq 0.05$.

Results

Physicochemical properties of soils

In our previous study, the physicochemical properties of paddy soil were found to be saline in nature and electric conductivity was 291 dS/m (Satapute and Kaliwal, 2016). Similarly, red loamy soil of the groundnut was having the pH of 6.7 and it was measured during the preparation of 1:1.25 slurry of water and soil. Additionally, the electric conductivity 0.202 dS/m (Table 1).

Table 1. Physicochemical properties of soil.

Nutrition	Red sandy loam soil
pH	6.7
Electric conductivity dS/m	0.202
Organic carbon %	0.72
Nitrogen kg/ha	201
Phosphorus kg/ha	92
Potassium kg/ha	583
Sulfur kg/ha	41.02
Calcium me/100g	31.9
Magnesium me/100g	5.01
Zinc (ppm)	0.76
Iron (ppm)	1.92
Magnesium (ppm)	0.30
Copper (ppm)	0.82
Sand (%)	50.21
Silt (%)	32.53
Clay (%)	18.93

Cellulase activity (EC 3.2.1.4)

Cellulase enzyme activity was increased in all the concentrations with the exception of 10 kg/ha and the impact of all the application rates of propiconazole on

the cellulase enzyme activity is illustrated in the Table 2.

Explaining the activity of the enzyme under desired application rates incubated for the 24 hrs extracted after 10 days.

Table 2. Activity of cellulase (*µg of glucose/gram) under the impact of propiconazole in red loamy and paddy soil for 24 hrs after 10 days.

Concentrations of propiconazole (kg/ha)	Propiconazole (Tilt)	
	Red loamy Soil	Paddy soil
0	345±2.6034 ^c	388±5.0442 ^f
1	373±7.0000 ^d	416±6.2271 ^d
2.5	503±7.0946 ^c	522±2.4037 ^c
5.0	647±2.3333 ^a	681±2.0816 ^a
7.5	530±5.5075 ^b	546±4.0414 ^b
10.0	340±3.3829 ^f	392±4.7022 ^e

All column is mean ± S.E. for respected concentrations in every group; column sharing the different letters (a, b, c, d, e and f) are significantly different with each other ($p \leq 0.05$).

It was also noticed that, the stimulatory activity of the cellulase enzyme in paddy and red loamy soil was found at the 5 kg/ha. Moreover, in both the type of soil cellulase activity was significantly increased. While comparing the obtained activity with control it was unveiled that, soil treated with the 1 and 2.5 Kg/ha of fungicide enhances the activity of cellulase after 10 days. In contrast, 7.5 and 10 kg/ha treated soil types were significantly decreases in the activity of

cellulase. On the other hand, the stimulatory application rate 5 kg/ha was induces activity of cellulase after the 10th days. Additionally, in 20th day the activity was found to be highest in the both paddy and red loamy soil. Whereas, on the 30th and 40th day, the activity was decreased and importantly the decline stage of enzyme activity in both the soils were considered to be between 30-40th days (Fig. 1A and 1B).

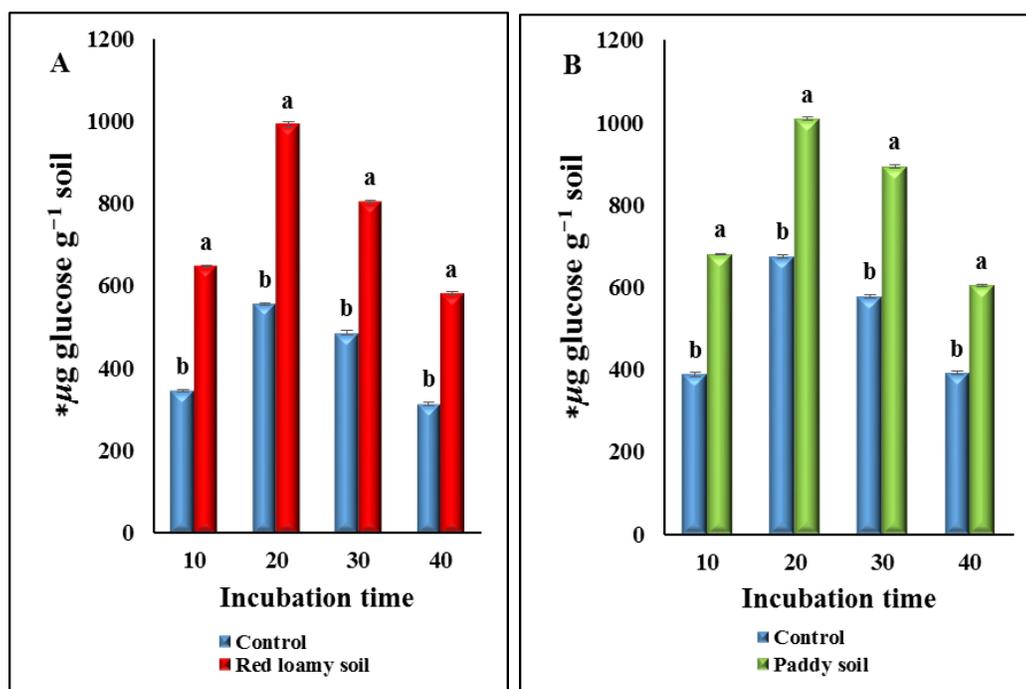


Fig. 1: Influence of propiconazole at 5.0 kg/ha on cellulase activity on Red loamy soil of ground nut (A) and paddy soil (B) *µg of glucose/gram soil formed after 24 hrs with 1% carboxy methyl cellulase after 10, 20, 30, and 40 days. The data are the means ± S.E. for each incubation period.

Invertase activity (EC 3.2.1.26)

In the present investigation of invertase activity in paddy and red loamy soils. Showed the induced increment in the concentration of invertase in application rates of 1 and 2.5 kg/ha. Whereas, the decline phase of enzyme activity was initiated from the 5 kg/ha, similarly in the 7.5 and 10 kg/ha the liberal reduction in the enzyme activity was found for 24 hrs and 48 hrs after 10 days of incubation. Moreover, the stimulatory impact was occurred at 2.5 kg/ha in both types of soils (Table 3 and 4). In comparison with control the progressive increment

of invertase activity was obtained at the rate of 1 and 2.5 kg/ha. But, the soil types treated with 10 kg/ha were significantly decreased in the enzyme activity than the control. On the other hand, the stimulatory application rate 2.5 kg/ha was induces activity of invertase after 10 days incubation period. Furthermore, in the 20th day the activity was found to be maximum in the both paddy and red loamy soil. However, after the 20 days of incubation the activity was lowered and it was important to note that, the decline period of invertase enzyme activity in the both soils were considered to be between 30-40th days (Fig. 2A and 2B).

Table 3. Activity of invertase (μg of glucose/gram) under the impact of propiconazole in red loamy and paddy soil for 24 hrs after 10 days.

Concentrations of propiconazole (kg/ha)	Propiconazole (Tilt)	
	Red loamy Soil	Paddy soil
0	799 \pm 2.4037 ^e	776 \pm 2.8480 ^e
1	894 \pm 4.0414 ^d	979 \pm 2.0816 ^c
2.5	1054 \pm 5.8972 ^a	1142 \pm 4.0960 ^b
5.0	1001 \pm 3.4801 ^b	1115 \pm 5.1747 ^a
7.5	970 \pm 4.9103 ^c	809 \pm 5.5075 ^d
10.0	770 \pm 4.3589 ^f	737 \pm 3.3829 ^f

All column is mean \pm S.E. for respected concentrations in every group; column sharing the different letters (a,b,c,d,e and f) are significantly different with each other ($P \leq 0.05$).

Table 4. Activity of invertase (μg of glucose/gram) under the impact propiconazole in red loamy and paddy soil for 48hrs after 10 days.

Concentrations of propiconazole (kg/ha)	Propiconazole (Tilt)	
	Red loamy soil	Paddy soil
0	910 \pm 3.7564 ^e	890 \pm 1.2018 ^e
1	1003 \pm 2.7284 ^c	993 \pm 2.403 ^c
2.5	1040 \pm 0.8819 ^a	1213 \pm 8.3266 ^a
5.0	1002 \pm 7.1248 ^b	1116 \pm 3.1798 ^b
7.5	970 \pm 1.5275 ^d	922 \pm 2.0000 ^d
10.0	830 \pm 1.6667 ^f	832 \pm 7.2188 ^f

All column is mean \pm S.E. for respected concentrations in every group; column sharing the different letters (a,b,c,d,e and f) are significantly different with each other ($P \leq 0.05$).

Discussion

Continuous and repeated application of pesticides in the agricultural fields and also to the standing plantations will lead to the rise of many problems associated with soil health, disturbance to the soil micro flora which will directly have the impact on microorganism's populations and enzymatic imbalance. Currently, many of the pesticide are used to control the diseases and pests for the better crop yielding, unfortunately, most of the desired pesticides are used heavy or more than recommended doses. It is well understood that, pesticides have the ability to limit the population of soil bacteria (Shetti and Kaliwal, 2012). Correspondingly,

pesticide stimulation on the enzyme activity and modulatory impact of pesticides have been previously reported by the number of researcher (Ismail et al. 1996; Sannino and Gianfreda, 2001).

Many of the experimental results of propiconazole persistent in soil and its toxicity have been reported. Singh and Dureja (2008) have shown a clear picture about the persistence of propiconazole in the soil. Additionally, the degradation rate of propiconazole was found slow in the paddy (Kim et al., 2002). Moreover, in vitro toxicity analysis of propiconazole on human cell lines authenticates its toxicity (Satapute and Kaliwal, 2015). Conversely, Yang et al. (2011) and

Niewiadomska and Maruwka (2011) have reported that, propiconazole will have the greatest impact to inhibit the bacterial activity in the wetlands.

In the present study, it was observed that cellulase activity in red loamy and paddy soil was significantly decreased at the higher concentrations (7.5 and 10 kg/ha) and in lower doses (1 and 2.5 kg/ha) the activity of cellulase has been gradually increased, importantly the stimulatory activity was found at the 5 kg/ha. However, the invertase activity was influenced by the

lower rate of concentrations (1 and 2.5 kg/ha). Meanwhile, the other rate of doses (5, 7.5 and 10 kg/ha) of propiconazole were affected the invertase activity. More importantly, the accumulation of glucose formed by the sucrose in paddy soil was observed more than the red loamy soil. Conversely, invertase activity was evidenced by the accumulation of glucose during the incubation period, it was also observed that the soil, which was incubated for the 48 hrs after 10 days showed higher activity of invertase than the 24 hrs incubation.

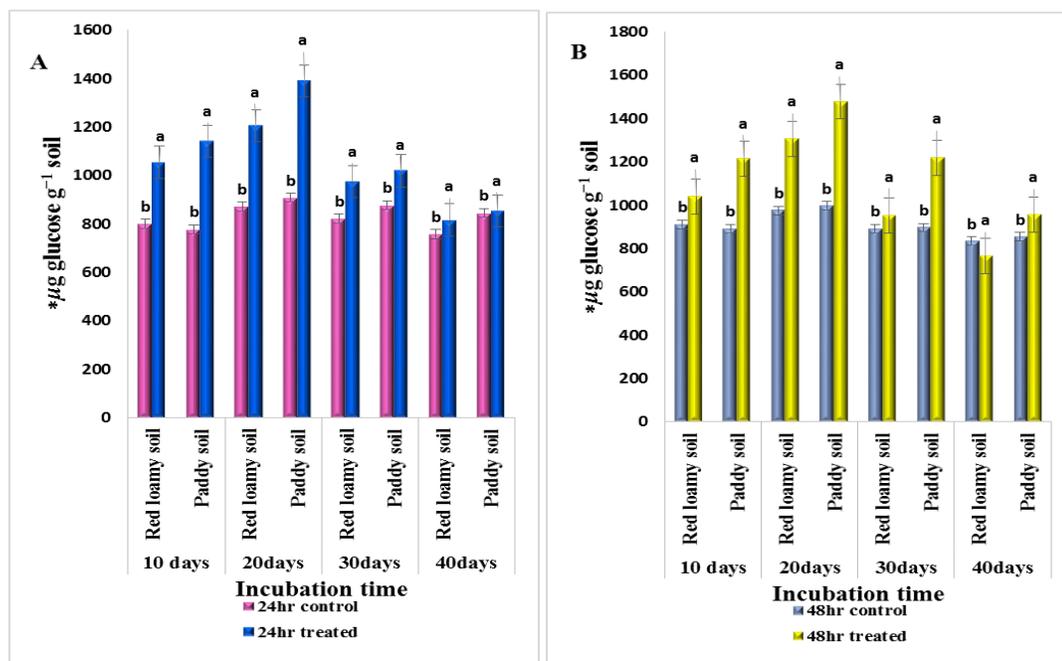


Fig. 2: Influence of propiconazole at 2.5 kg/ha on invertase activity in red sandy loamy soil of ground nut and paddy soil after 24 hrs (A) and 48 hrs incubation (B) *µg of glucose/gram soil formed with 18 mM sucrose after 10, 20, 30, and 40 days. The data are the means ± S.E. for each incubation period.

In support of our present study, Ramudu et al. (2011) have reported the similar trend of results explaining the impact of propiconazole on the ground nut soil enzyme activity, it was stated that the activity of cellulase and invertase was influenced by the accumulation of glucose. In the meantime the invertase activity was found higher than cellulase and prolonged incubation (40 days) soil treated with propiconazole does not show the stimulation and no quantifiable effect on the activity of enzymes. In contrast, the stimulatory impact of propiconazole on paddy soil was not investigated as is in the case of our study. The stimulatory application rate of propiconazole was found effective for the enzyme activity enhancement and it is proven in the present experimental results. Conversely, propiconazole is

mainly used in the paddy fields (Yen et al., 2009). While associating the results of the present investigation to other literatures, a similar trend for the cellulase and invertase activity were seen, the activity of both the enzymes were influenced by the accumulation of glucose but an investigation on paddy soil was rarely under represented. However, similar observations were reported on the impact of endosulfan and quinalphos on paddy soil enzyme activity (Vijaya et al., 2012). Furthermore, our results seemed to be reliable with previously published literatures (Srinivasulu and Rangaswamy, 2006; Mohiddin et al., 2010). Although, carbendazim and chloramphenicol were found toxic to the invertase at the rate of 8 mg/kg (Hu et al., 2011). Contradictory observations have been reported by

Mohiddin et al. (2013) saying that cellulase activity in red sandy loamy soil was decreased in the 5 kg/ha by the effect flubendiamide and the stimulatory activity was found at the 2.5 kg/ha. Whereas, present results evidenced by the increase of cellulase activity at 5 kg/ha. Although, a similar trend of the decline phase of cellulase and enzyme activity was reported. Decline in the activity of cellulase is mainly because of propiconazole toxicity and such conditions will have the impact on soil microorganisms. Previously, comparable observations on cellulase activity were reported by the Katayama and Kuwatsuka (1991) and Madhuri and Rangaswamy (2002).

Conclusion

The results of the current investigation would draw attention on the propiconazole toxicity on cellulase and invertase activity. Cellulase activity was greatly increased in 5 kg/ha followed by the 2.5 kg/ha of propiconazole concentrations, but the naturally dropping down of cellulase activity was observed at the 7.5 and 10 kg/ha in ground nut and paddy soil. When comparing the control in ground nut and paddy soil, invertase activity was shown the modulatory impact at 2.5 kg/ha. However, the activity was dramatically decreased at the 5, 7.5 and 10 kg/ha doses of propiconazole. Overall, propiconazole at recommended application rates (1, 2.5 and 5 kg/ha) will stance as healthier to soil enzymes and also to the soil microorganisms, more importantly the activity of both cellulase and invertase activity was higher in paddy soil than the red loamy soil of ground nut. Therefore, it indicates the microbial activity in the paddy soil is profoundly higher in the recommended doses.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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