

## Effect of the fungicide Mancozeb at different application rates on enzyme activities in a silt loam soil of the Kashmir Himalaya, India

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**Abstract:** Soil microbial diversity is indispensable to maintain functional diversity and enzyme-mediated critical soil processes that detoxify soil from environmental pollutants, like pesticides. Thus, the present study was carried out to assess the effect of different concentrations of the fungicide Mancozeb on the activities of alkaline phosphatase, protease, urease, amidase and asparaginase in silt loam soil of orchards of Kashmir Himalaya, India. In comparison to untreated control, application of ten times the recommended concentration ( $N_{10}$ ) of pesticide increased soil phosphatase activity by about 41 % after 14 days of incubation. However, after 28 days of incubation activity declined by about 30 % under different pesticide application treatments. Pesticide-induced stimulation of protease activity was recorded up to 21 days of incubation and thereafter a 17 % decrease in activity was observed in comparison to untreated control. Though all the pesticide application treatments had an inhibitory effect on urease activity, the  $N_{100}$  treatment had more consistent inhibitory effect. Amidase activity was stimulated up to 21 days of incubation irrespective of the concentration of pesticide used. Asparaginase activity, however, was reduced from 12 to 72 % in comparison to control for most of the incubation period. Microbial activity, evaluated through measurement of dehydrogenase activity, was stimulated in response to different concentrations of the fungicide.

**Resumen:** La diversidad microbiana del suelo es indispensable para mantener la diversidad funcional y los procesos edáficos críticos mediados por enzimas que desintoxican el suelo de sus contaminantes ambientales como los pesticidas. El presente estudio se llevó a cabo para evaluar el efecto de diferentes concentraciones del fungicida Mancozeb sobre las actividades de las enzimas fosfatasa alcalina, proteasa, ureasa, amidasa y asparaginasa en un suelo franco limoso de huertos de Kashmir Himalaya, India. En comparación con el control (sin tratamiento), la aplicación de diez veces la concentración recomendada ( $N_{10}$ ) de pesticida incrementó la actividad de la fosfatasa en el suelo en alrededor de 41 % después de 14 días de incubación. Sin embargo, después de 28 días de incubación la actividad declinó en alrededor de 30 % bajo diferentes tratamientos de aplicación del pesticida. La estimulación que indujo el pesticida en la actividad de la proteasa fue registrada hasta los 21 días de incubación y a partir de entonces se observó un decremento de 17 % en la actividad en comparación con el control. Aunque todos los tratamiento con aplicación del pesticida tuvieron un efecto inhibitorio en la actividad de la ureasa, el tratamiento  $N_{100}$  tuvo un efecto inhibitorio más consistente. La actividad de la amidasa fue estimulada hasta los 21 días de incubación independientemente de la concentración de pesticida usada. La actividad de la asparaginasa, sin embargo, se redujo de 12 a 72 % en comparación con el control durante la mayor parte del período de incubación. La actividad microbiana, evaluada a través de la medición de la actividad de la deshidrogenasa, fue estimulada en respuesta a diferentes concentraciones del fungicida.

**Resumo:** A diversidade microbiana do solo é indispensável para manter a diversidade funcional e mediação enzimática de processos críticos no solo, necessária para a desintoxicação dos mesmos em relação a poluentes ambientais como os pesticidas. Assim, o presente estudo foi realizado para avaliar o efeito de diferentes concentrações do fungicida Mancozeb sobre as

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actividades da fosfatase alcalina, protease, urease, amidase e asparaginase em solo franco argiloso em pomares na Caxemira, Himalaias, Índia. Em comparação com a testemunha, a aplicação de dez vezes a concentração recomendada (N<sub>10</sub>) de pesticida aumentou a actividade da fosfatase do solo por cerca de 41 % após 14 dias de incubação. Contudo, após 28 dias de incubação a actividade diminuiu cerca de 30 % sob os diferentes tratamentos de aplicação de pesticida. A estimulação induzida pelo pesticida na actividade da protease foi registada até 21 dias de incubação e, depois disso, verificou-se uma redução de 17 % naquela actividade em comparação com a testemunha. Embora todos os tratamentos de aplicação de pesticidas tenham um efeito inibitório na actividade da urease, o tratamento N<sub>100</sub> teve um efeito inibitório mais consistente. A actividade da amidase foi estimulada até 21 dias de incubação, independentemente da concentração do pesticida usado. A actividade da asparaginase, no entanto, foi reduzida de 12 para 72 % em comparação com o controlo na maior parte do período de incubação. A actividade microbiana, avaliada através da medição da actividade de desidrogenase, foi estimulada em resposta a diferentes concentrações do fungicida.

**Key words:** Microbial activity; pesticide use; soil microbial enzymes.

## Introduction

Arable land is often amended with agrochemicals like, fertilizers and pesticides to increase agricultural productivity, and such practices are an integral part of modern agriculture. In a country like India, the greatest challenge in improving agricultural productivity still remains the curtailment of crop losses due to pests, estimated at about 50 % of total food production and 20-30 % of sown crops valued at Rs. 900 billion per annum (Anonymous 2002). The Jammu and Kashmir state of India is a major producer of apple and has an annual production of 1 million tonnes. With an annual turnover of over Rs. 3 billion excluding the foreign exchange of over Rs. 800 million, horticulture plays a vital role in the economic development of the state. However, significant yield losses in apple production due to apple scab, caused by the fungus *Venturia inaequalis*, are a major concern. The fungicide Mancozeb, in addition to other pesticides, is extensively used in apple orchards for control of apple scab, but it has the potential to affect the quality of soil, water and air, with attendant risk to humans, flora and fauna, mainly due to its persistence in soil (Huber *et al.* 2001; Munshi 1986).

Because of their relationship to soil biology and rapid response to changes in soil management, soil enzymes are recognized as sensitive indicators of soil health and quality (Bandick & Dick 1999; Caldwell 2005; Dick *et al.* 1996). In fact, they have

been related to soil physico-chemical characters (Amador *et al.* 1997), microbial community structure (Kourtev *et al.* 2002), and disturbance (Boerner *et al.* 2000). With respect to pesticides, however, so little has been done in so few locations that broad generalizations can not be drawn (Schäffer 1993). Thus, the present investigation was aimed to specifically focus on the effects of mancozeb at different application rates on some key enzyme activities involved in nitrogen, and phosphorus cycling in a silt loam soil which is commonly found in orchards of the Kashmir Himalaya.

## Materials and methods

### *Pesticide application*

Mancozeb [[1,2-ethanediybis-[carbamodithioato]] (2-) manganese, mixture with [[1,2-ethanediybis-[carbamodithioato]]-(2-)] zinc, is a fungicide of the carbamate pesticide family. In the Kashmir Himalayan apple orchards, Mancozeb is sprayed at pink bud, petal fall and pre-harvesting stages at a rate of 30 kg ha<sup>-1</sup> per spray. In addition to this usual (normal) application rate, higher pesticide concentrations were also included in the experimental set up so as to predict its likely impact on the activity of soil enzymes in the event of its excessive use and continuous build up in the soil. The application rates used in the present study included:

- (a) Normal application rate of 60 kg ha<sup>-1</sup> (N)
- (b) 10 times the normal application rate (N<sub>10</sub>)

- (c) 100 times the normal application rate (N<sub>100</sub>)  
 (d) Untreated control (C)

Conversion of field application rates to mg of pesticide per gram air dried soil was made assuming a uniform distribution of the chemical in top 0-10 cm of soil having bulk density of 1.3 g cm<sup>-3</sup>. Soil was maintained at 60 % of maximum water holding capacity using distilled water. In each of the treatments the pesticide was thoroughly mixed with soil and three replicates of treated and untreated (control) soil samples were incubated at 25 ± 1 °C in the dark. At periodic intervals of incubation, three replicate soil samples were recovered for estimating microbial activity and soil enzyme activity. Soil type used in the present study was a silt loam (clay = 28 %, silt = 50 % and sand = 22 %) with pH 7.5 and 1.6 % organic carbon.

#### Soil enzyme assays

Dehydrogenase activity (DHA) was determined according to Casida *et al.* (1964) as an index of microbial activity. The method relies on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) by microorganisms to 2,3,5-triphenyl formazan (TPF). The concentration of TTC was measured on a spectrophotometer (Systronics, Model-106) at 485 nm using methanol as blank.

Activity of alkaline phosphatase was assayed according to Tabatabai & Bremner (1969) and Eivazi & Tabatabai (1977) using p-nitrophenyl phosphate solution as the substrate. Clear yellow coloured solution formed as a result of the action of alkaline phosphatase on the substrate was analysed spectrophotometrically at wavelength of 410 nm to measure the amount of p-nitrophenol released.

Soil protease activity was determined with the method outlined by Ladd & Butler (1972) and modified by Rangaswamy *et al.* (1994) and Ismail *et al.* (1996). The amino acids released by protease action on sodium caseinate (substrate) were quantified by reference to calibration graph drawn using absorbance values of tyrosine standards.

Activities of urease (Tabatabai & Bremner 1972), amidase (Frankenberger & Tabatabai 1980a,b) and asparaginase (Frankenberger & Tabatabai 1991) were determined using urea, formamide and L-asparagine as the substrates and the amount of NH<sub>4</sub><sup>+</sup>-N released by action of each of these enzymes on their respective substrates was determined by steam distillation.

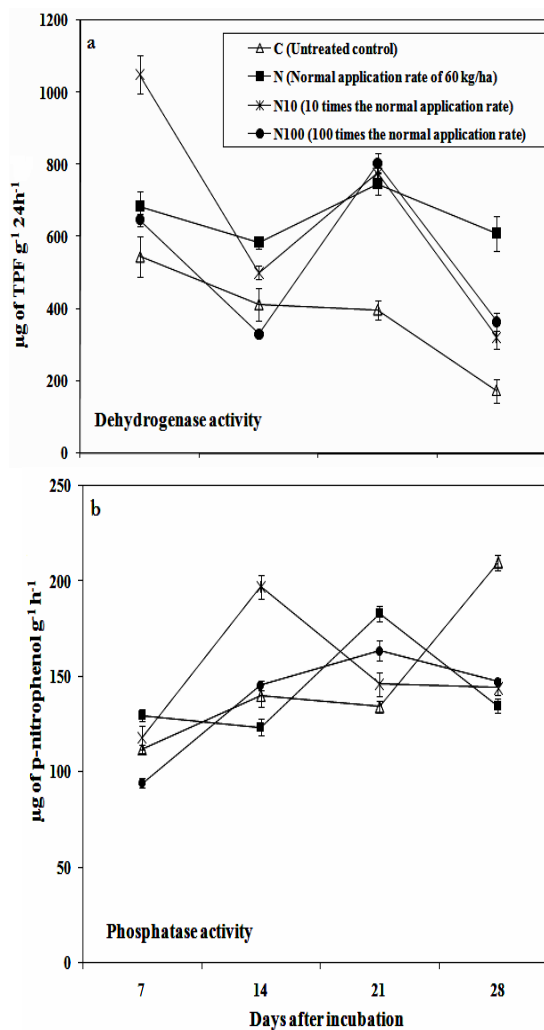
#### Statistical analysis

The data were statistically analyzed using a two-way analysis of variance (ANOVA) with SPSS 10 statistical software (SPSS Inc., USA 1999).

## Results

#### Dehydrogenase activity

The effect of different application rates of mancozeb on dehydrogenase activity is presented in Fig. 1a. After 7 days of incubation microbial



**Fig. 1.** Effect of different application rates of Mancozeb on: (a) soil dehydrogenase, and (b) Soil phosphatase activities (Data points are means of three replicates ± S.E.).

activity increased under all the treatments compared to the control. The stimulatory effect was particularly prominent for the N<sub>10</sub> treatment and the lowest values of activity were detected for the control. In comparison to the control, N<sub>100</sub> treatment induced the highest microbial activity after 14 days of incubation. After 21 days of incubation all the pesticide treatments had similar enzyme activity and continued to be higher compared to the control. Even after 28 days of incubation microbial activity remained higher in the pesticide-treated soil samples than in the control.

#### *Alkaline phosphatase activity*

Alkaline phosphatase activity (Fig. 1b) revealed a variable pattern in response to different pesticide concentrations. For example, N<sub>10</sub> brought about a 41 % stimulation in activity after 14 days of incubation compared to the control, but after 28 days of incubation a 30 % decrease in enzyme activity was recorded in response to all pesticide concentrations.

#### *Protease activity*

Pesticide applications had a stimulatory effect on protease activity in comparison with the control up to 21 days of incubation (Fig. 2a). In particular, the N<sub>100</sub> treatment enhanced protease activity (27 %) much more than other treatments. However, the same pesticide concentration resulted in 17 % decrease in protease activity after 28 days of incubation relative to the control.

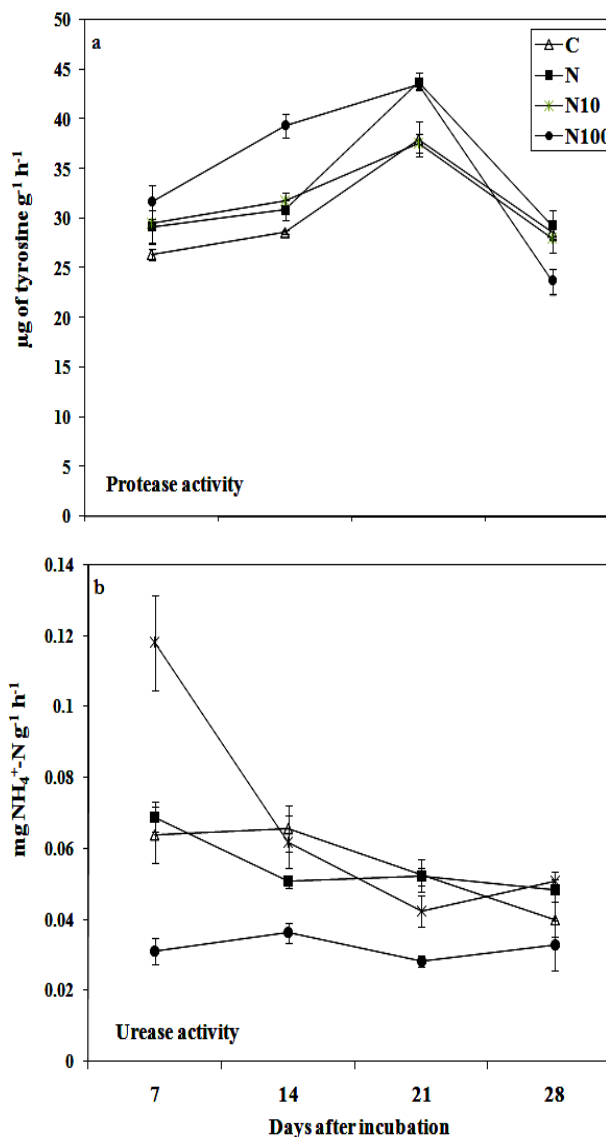
#### *Urease activity*

Urease activity was inhibited consistently by the N<sub>100</sub> treatment during the study (Fig. 3b), with maximum inhibition (52 %) recorded after 7 days of incubation. However, the inhibitory effect was reduced upon further incubation. Compared to the control, the N<sub>10</sub> treatment stimulated urease activity after 7 days of incubation, followed by decline and a marginal recovery after 28 days.

#### *Amidase activity*

Amidase activity was positively influenced by the fungicide treatments throughout the period of incubation (Fig. 3a). A sharp increase in activity occurred after 14 days of incubation, with the highest increase (99 %) occurring under the N<sub>100</sub> treatment. Following this increase, a significant decline was recorded after 21 days of incubation. After 28 days of incubation the N and N<sub>10</sub> treat-

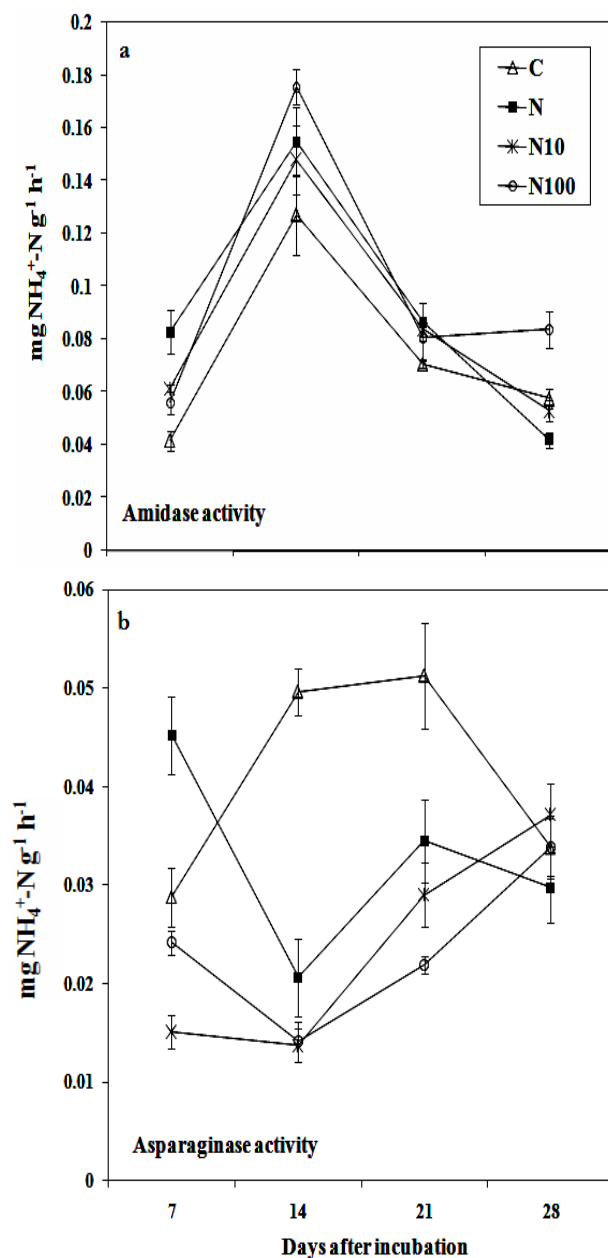
ments inhibited amidase activity by 37 % and 8 %, respectively, whereas N<sub>100</sub> still had a stimulatory effect on enzyme activity.



**Fig. 2.** Effect of different application rates of Mancozeb on: (a) Soil protease, and (b) Soil urease activities.

#### *Asparaginase activity*

Except for an initial significant stimulation for the N treatment (Fig. 3b), asparaginase activity was reduced between 12 and 72 % in response to different pesticide concentrations. However, the activity recovered after 28 days of incubation, and the N<sub>10</sub> treatment had a marginal stimulatory effect on the enzyme activity.



**Fig. 3.** Effect of different application rates of Mancozeb on: (a) Soil amidase, and (b) soil asparaginase activities.

## Discussion

Pesticide concentration, period of incubation and their interaction significantly ( $P < 0.05$ ) affected the activity of different soil enzymes (Table 1). Dehydrogenase activity, used as an index of physiologically-active microorganisms in

soil (Demanou *et al.* 2004; Nannipieri *et al.* 1990; Tabatabai 1994) increased in response to different pesticide treatments, but the extent of increase varied significantly across different treatments (Table 1). Use of the pesticide as a source of carbon and energy could explain the increased DHA activity in soil samples treated with the pesticide. Differences in dehydrogenase activity under different pesticide treatments may be ascribed to differences in the decomposition rates of pesticides, or to differential effects of different pesticide concentrations on the soil microbial community, as suggested by Monkiedje & Spiteller (2002) and Nannipieri & Bollag (1991).

**Table 1.** Results of two-way analysis of variance showing effect of pesticide concentration (treatments) and days of incubation on the activity of different soil enzymes (Data represent F-values significant at  $P < 0.05$ ).

Dependent variable	Independent variable		
	Pesticide concentration (PC)	Days of incubation (DI)	PC × DI
Dehydrogenase activity	60.42	107.13	17.49
Phosphatase activity	8.31	100.48	49.96
Protease activity	8.49	90.81	6.83
Urease activity	27.86	19.93	7.62
Amidase activity	7.31	28.03	3.47
Asparaginase activity	27.13	8.39	11.22

Because of their involvement in making phosphate available to crop plants (Somerville & Greaves 1987), soil phosphatases have been extensively studied in relation to pesticide use. Pesticide treatments (Fig. 1a) had no consistent effect on alkaline phosphatase activity (Fig. 1b), with stimulation under N and N<sub>10</sub> and inhibition under N<sub>100</sub> application rates. After 28 days of incubation, enzyme activity was highest under untreated control conditions compared with pesticide treatments. Such a pattern suggests that the pesticide itself may not be inhibitory to phosphatase activity, but rather its degradation products may be responsible for inhibition, since these might become available towards the latter stages

of incubation, when inhibition was observed. Variable responses vis-à-vis pesticide concentrations, as observed during the present study (Table 1), have been reported by other workers as well. Although, Atlas *et al.* (1978) reported no change in soil phosphatase activity enzyme activity in response to application of folpet and captafol, Tarafdar (1986) observed a decrease in phosphatase activity due to fluchloralin, methabenzthiazuron, metoxuron, 2,4-D and isoproturon applied at recommended field rates. Contrarily, stimulation in phosphatase activity under the influence of paraquat, trifluralin, glyphosate and atrazine has been reported by Hazel & Greaves (1981).

Soil protease activity, known to be of importance in soil nitrogen cycling (Kamimura & Hayano 2000), was stimulated up to 21 days of incubation, followed by a sharp decline after 28 days, particularly under N<sub>100</sub> treatment. Contrary to results obtained during the present study, phenmedipham at 10 mg kg<sup>-1</sup> did not affect protease activity (Maško *et al.* 1991). However, stimulation of protease activity in a native soil was reported after treatment with linuron at 10 mg kg<sup>-1</sup>, whereas, cartap-HCl at 100-1000 mg kg<sup>-1</sup> inhibited the enzyme activity without any recovery during a period of 60 days (Endo *et al.* 1982).

Urease has been studied more extensively relative to other soil enzymes because of its involvement in the breakdown of urea, a commonly used fertilizer (Martens & Bremner 1997). In the present study, N<sub>100</sub> treatment of Mancozeb brought about inhibition of urease activity (Fig. 2b) but stimulation in the enzyme activity was recorded under N<sub>10</sub> treatment. While the inhibition of urease activity could be due the presence of Mn and Zn ions in the pesticide, as reported by Tabatabai (1977), stimulation may be due to the role of Mancozeb as a source of nutrients to soil microorganisms.

Since aminohydrolases (amidase and asparaginase) catalyse the hydrolysis of C-N bonds other than the peptide bonds (Dodor & Tabatabai 2003), they play a major role in soil organic N mineralization. During the present study, Mancozeb at all the application rates stimulated amidase activity; however, towards the latter stages a slight inhibition was noticed in N and N<sub>10</sub> treatments. Earlier reports that thiol groups are not present in the enzyme's active site (Frankenberger & Tabatabai 1991) are also borne out by the present finding of lack of inhibition of amidase activity by Mn<sup>2+</sup> and Zn<sup>2+</sup> containing Mancozeb. Except for an

initial stimulation under the normal application rate, asparaginase activity was inhibited irrespective of the rate of application of Mancozeb used in the present study.

The results of the present study suggest that pesticide application affects activities of different soil enzymes differently. While the activities of urease and asparaginase were generally inhibited, dehydrogenase, protease and amidase activities were stimulated in response to pesticide treatments. Phosphatase activity, however, exhibited a highly variable response to different concentrations of pesticide used during the present study. Thus, a judicious use of Mancozeb for control of apple scab is recommended so as to prevent adverse impacts on the biology and biochemistry of the soil.

### Acknowledgement

We wish to thank Head, Department of Botany, University of Kashmir, Srinagar for providing necessary laboratory facilities.

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