Journal of Academia and Industrial Research (JAIR)
Volume 10, Issue 3, Pages 50-54



ISSN: 2278-5213

Research Article

# Activities of Dehydrogenase and Protease in Cotton Cultivated Black Soils as Influenced by Fungicides

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Received: February 25 2022/Accepted: April 28 2022/Published: 07 May 2022

## Abstract

The effects two fungicides mancozeb and hexaconazole on the activities of dehydrogenase and protease in soil, collected from cotton cultivated black soils of Kurnool District of Andhra Pradesh, India was studied. The influence of fungicides on the enzyme activities increased with increasing concentrations of the fungicides up to 2.5 kg $ha^{-1}$ and 5.0 kg $ha^{-1}$ which was dose-dependent. Higher rates beyond 5.0 kg $ha^{-1}$ of the fungicides were either mild or toxic to the enzyme activities. The significant stimulation in the activity of dehydrogenase was more at 2.5 kg $ha^{-1}$, whereas the activity of protease was significantly increased at 5.0 kg $ha^{-1}$, lasted up to 21days and 30 days respectively. Results obtained from the data in the present study evidently specify that the fungicides, widely used in cultivation of cotton, at field application rates boost the activities of dehydrogenase and protease in black soil.

**Keywords:** Black cotton soil, fungicides, mancozeb, hexaconazole, dehydrogenase, protease.

## Introduction

Cotton (Gossypium spp.), referred as 'King of Fibre' and 'White Gold', is the most extensively cultivated commercial crop which plays a key role in economic development. In India, cotton was cultivated in an area of 122.38 lakh ha with an annual production of 361 lakh bales of 170 kg and a productivity of 501 kg lint/ha during 2018-19. Andhra Pradesh stood 4th in area (6.66 lakh $ha^{-1}$) but $7^{th}$ in production (20.0 lakh bales) and 5th in productivity (617 kg/ha) (Annual Report, ICAR-All India Coordinated Research Project on Cotton, Coimbatore, Tamil Nadu, India, 2019). However, the area under cotton for the year 2021-22 was 32.10 million hectares, production and productivity accounted for 257.71 million bales and 1370 kg/ha respectively globally. India has emerged as the largest producer of cotton in the world and occupies the first position in terms of both total area and production (ANGRAU, 2021). Soils and climate in Andhra Pradesh are conducive for growing a variety of crops. The dominant crops are Rice, Groundnut, cotton, Jowar, Maize, Green gram, Black gram and Red gram. The cotton crop stands in the third place in terms of area. In India, there are nine major cotton growing states which fall under three zones, viz. the North Zone (Punjab, Haryana and Rajasthan), the Central Zone (Maharashtra, Madhya Pradesh and Gujarat), and the Southern Zone (Andhra Pradesh, Karnataka and Tamil Nadu). Nearly 65% of the cotton crop is cultivated under rainfed conditions in the country. Nearly $2/3^{rd}$ of the Cotton production in India comes from the states of Maharashtra, Gujarat, Andhra Pradesh, and Telangana, collectively known as the Cotton Basket of India (Gandhi and Jain 2006; ANGRAU, 2021). Different classes of fungicides are used in agriculture to control fungal diseases. Impact of these fungicides is beneficial in improving agricultural productivity by the control of pests and diseases, but a major portion of these agrochemicals tend to affect the soil biological activity in different ways (Ramakrishna et al. 1997, Nagaraj *et al.*, 1997). By the extensive and repeated application of pesticides year after year, they finally reach the soil, which may in turn interact with soil microorganisms and their metabolic activities. According to Anderson (1978) and Andreas *et al.* (2000), the behavior of the total microflora and their biological activity (enzyme activities) under continued fungicide input are important aspects of research in the ecology of agricultural field. Many enzymes like proteases, dehydrogenases, amylases, cellulases, urease etc., are influenced by fungicides. Soil dehydrogenase has a role in the initial stage of oxidation of soil organic matter (Tu 1980, Ajungla *et al.*, 2003). Proteases are oldest enzymes known to man, which are involved in the initial hydrolysis of the protein components of the organic nitrogen to simple amino acids in soils. It has been shown that proteases in soil can hydrolyze not only added proteins but also native soil

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proteins (Dedeken and Voets, 1965; Kiss *et al.,* 1975; Raju *et al.,* 1994). An ideal xenobiotic must be toxic to only target organisms, totally biodegradable and able to not leaving any intermediary compounds in the environment or being lixiviated to underground waters as reported by Ros *et al.* (2006). Pesticides may influence more or less soil biological activities, which is the result of microbial and enzymatic transformations and may be toxic to some important bacterial groups where as other microorganisms are able to use some pesticides as energy and nutrient sources (Johnsen *et al.,* 2001; Monkiedje *et al.,* 2002). In view of the above, the current work attempts to focus on the effects of two fungicides viz., mancozeb, hexaconazole on protease and dehydrogenase activities of the soil collected from cotton cultivated fields of Kurnool district.

## Materials and Methods

***Soil:*** Soil sample (black cotton) was collected from cotton grown fields of Kurnool district, up to a depth of 15 cm, and were air-dried and sieved through 2 mm sieve before use. The physico-chemical characteristics of these soils were determined as presented in Table 1.

***Fungicides:*** Samples of two fungicides, mancozeb and hexaconozole were dissolved in acetone. Details of the fungicides used in the present study are shown in Table 2.

**Soil incubation**: Aliquots (0.05 mL) from stock solutions of the fungicides, prepared in acetone, were applied with 0.1mL pipette to the surface of 10 g soil samples contained in test tubes (25 mm x 200 mm) as followed by Lethbridge and Burns (1976). The final concentrations (on w/w basis) of each fungicide included 10, 25, 50, 75 and 100 pg soil, which correspond to 1.0, 2.5, 5.0, 7.5 and 10 kg $ha^{-1}$ (Anderson 1978). The field application dose of the selected fungicides ranged from 2.5 to 5.0 kg $ha^{-1}$ (Anonymous, 1988). The soil samples receiving only 0.05 mL acetone served as controls. After complete evaporation, all the treatments including controls were maintained at 60% water holding capacity (WHC), and incubated in the laboratory at 28±4°C. After seven or 10 days of incubation, soil samples in duplicate were withdrawn for the assay of dehydrogenase (Chendrayan and Sethunathan, 1980) and protease (Speir and Ross, 1975). In another experiment, the soil samples were treated with only 2.5 and 5.0 kg $ha^{-1}$, of the two fungicides. Soil samples with no fungicide treatment served as controls. Soil moisture was maintained at 60% WHC and incubated in the laboratory at 28±4°C. Moisture levels were restored to their initial values during incubation. Duplicate soil sample of each treatment including the controls were withdrawn after 7, 14, 21, 28 and 35 days, and 10, 20, 30 and 40 days of incubation, for the assay of dehydrogenase and protease respectively.

***Enzyme activity:*** The activities of protease and dehydrogenase under the influence of two fungicides at different concentrations were determined in black cotton soil. Five grams of dried black soils were transferred into test tubes (12 x 125 mm) and treated with different concentrations of insecticides and fungicides to provide final concentrations of 10, 25, 50, 75 and 100 µg g-1 soil (equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha-1 field application rates). The soil samples without pesticides treatment served as control. The water holding capacity (WHC) of soil was maintained at 60% by 2 mL adding sterilized distilled water to the test tubes containing soil. All the treatments including controls were maintained in the laboratory at 28±4°C for 7, 14, 21, 28 and 35 days. During incubation period certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the enzyme assay.

***Assay of dehydrogenase:*** The method used for the assay of dehydrogenase was done according to Casida *et al.* (1964), Rangaswamy *et al.* (1994) and Sreenivasulu *et al.* (2013). This method is based on the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). Each soil sample was treated with 0.1 g of $CaCO_3$ and 1 mL of 0.18 mM aqueous solution of TTC and incubated for 24 hrs at 30°C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a UV Visible Spectrophotometer (Thermo Scientific Evolution 201). Dehydrogenase activity was measured at 7, 14, 21, 28 and 35 days of incubation.

***Assay of protease:*** Untreated and fungicide-treated soil samples (2g) were incubated for 24 h at 30°C with 10 mL of 0.1M tris (2-amino-2 (hydroxyl methylmethyl)-propane-1:3 diol, -pH 7.5) containing sodium caseinate (2% w/v). Four mL of aqueous solution (17.5% w/v) of trichloroacetic acid was then added and the mixture was centrifuged. Suitable aliquots of the supernatant were treated with 3 mL of 1.4M NaCO3, followed by the addition of 1.0 mL Folin-Cicalteu reagent (33.3% v/v). Using tyrosine as a standard the blue color obtained was read after 30 min at 700 nm in a spectrophotometer.

***Statistical analysis:*** The concentration of the enzyme activity was calculated on a soil weight (oven dried) basis. The fungicide treatments were contrasted with untreated controls and the significant difference ($P<0.05$) between values of each sampling and each fungicide were performed using Duncan's new multiple range (DMR) test (Rangaswamy *et al.,* 1989).

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Table 1. Physico-chemical properties of black cotton soils used in the present study.

| Properties | Cultivated soil | Uncultivated soil |
|---|---|---|
| Texture | Clayey | Clayey |
| Sand (%) | 20.7 | 21 |
| Silt (%) | 23.8 | 24.5 |
| Clay (%) | 55.5 | 54.5 |
| pHa | 8 | 8.4 |
| Water holding capacity (ml g-1 soil) | 0.27 | 0.29 |
| Electrical conductivity (m.mhos) | 265 | 255 |
| Organic matter b (%) | 1.48 | 1.56 |
| Total nitrogen c (%) | 0.086 | 0.091 |
| NH4+ - N (µg g-1 soil) d | 8.11 | 8 |
| NO2- -N (µg g-1 soil) e | 0.38 | 0.36 |
| NO3- - N (µg g-1 soil) f | 0.79 | 0.76 |

Where, a = 1:1.25=Soil: Water slurry; b = Walkley-Black Method (Jackson, 1971): c =Micro-Kjeldahl Method (Jackson, 1971); d= Nesslerization method (Jackson, 1971); e = Diazotization Method (Brnes and Folkard, 1951); f = Brucine Method (Ranney and Bartlett, 1972).

Table 2. Properties of the fungicides used in the present study.

| Technical name | Commercial name | Chemical Class | Commercial purity | Source of samples |
|---|---|---|---|---|
| Mancozeb | Dhanuka M-45 | Dithiocarbamate | 75%WP | Northern Minerals Ltd. Gurgaon |
| Hexaconazole | Hexadhan | Triazoles | 5% EC | Dhanuka Agritech Ltd. |

Table 3. Activity of dehydrogenase under the impact of different concentrations of selected fungicides in black soils for 24 hours after 7 days.

| Concentration of fungicides (Kg $ha^{-1}$) | Mancozeb | Hexaconazole |
|---|---|---|
| 0.0 | 48a | 48a |
| 1.0 | 84b | 72b |
| 2.5 | 200c | 181c |
| 5.0 | 150d | 120d |
| 7.5 | 110e | 60e |
| 10.0 | 40f | 36f |

Means in each column followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to DMR test.

## Results and Discussion

In the present study, soil dehydrogenase, one of the indexes of microbial activity, measured in terms of triphenyl Formazen accumulated from TTC, has been employed as this enzyme is affected by addition of fungicides (Cervelli *et al.*, 1978). Concentrations of Mancozeb and Hexaconazole caused significant stimulation in enzyme activity during 7 day incubation in soil samples ranged from 1 to 7.5 kg $ha^{-1}$ (Table 3). The accumulation of formazen was more conspicuous at the 2.5 kg $ha^{-1}$ level in case of dehydrogenase, whereas in case of protease it was 5.0 kg $ha^{-1}$.

The extent of dehydrogenase activity of soil samples under the impact of selected fungicides at 2.5 kg $ha^{-1}$ was also determined after incubating the fungicide treated soil samples for 7, 14, 21, 28 and 35 days (Table 4). As reported by Chendrayan and Sethunathan (1980) the dehydrogenase activity was relatively less in the soil maintained under non-flooded conditions in general. This can be anticipated because dehydrogenase activity is significantly more pronounced in flooded soils, as most dehydrogenases are of anaerobic origin. There was a progressive increase in the accumulation of formazan with increasing period of incubation up to 21 days, which gradually decreased further. Hence, the dehydrogenase activity was enhanced significantly more at 2.5 kg $ha^{-1}$ of the two fungicides. However, application of fungicides to soils led to an initial increase in dehydrogenase activity. By the end of 5 weeks of soil incubation, fungicide application had virtually no influence on the enzyme activity. The soil samples, treated with concentrations ranging from 1 to 10 kg $ha^{-1}$ of the fungicides, and incubated for 10 days, were supplemented with 1% casein in order to determine the non-target effects of these fungicides on protease activity, measured in terms of tyrosine formed after 24 hours at 30°C. Application of Mancozeb up to 5 kg $ha^{-1}$ or Hexaconazole up to 7.5 kg $ha^{-1}$, greatly enhanced the activity of protease in soil (Table 5). The activity of protease was significantly more pronounced in soil samples that received 5.0 kg $ha^{-1}$ of the two fungicides. Protease activity was enhanced significantly at 5.0 kg $ha^{-1}$ until 30 days of soil incubation (Table 6).

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Table 4. Influence of selected fungicides at 2.5 kg ha$^{-1}$ on dehydrogenase activity in black soils.

| Name of the fungicide | 7 days | 14 days | 21 days | 28 days | 35 days |
|---|---|---|---|---|---|
| Control | 48a | 80a | 124a | 106a | 65a |
| Mancozeb | 200b | 380b | 478b | 221b | 136b |
| Hexaconozole | 180c | 368c | 462c | 201c | 130b |

Means in each column followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to DMR test.

Table 5. Activity of protease under the impact of different concentrations of selected fungicides in black soils for 24 hours after 7 days.

| Concentration of fungicides (Kg ha$^{-1}$) | Mancozeb | Hexaconazole |
|---|---|---|
| 0.0 | 422a | 421a |
| 1.0 | 585b | 543b |
| 2.5 | 673c | 564c |
| 5.0 | 748d | 665d |
| 7.5 | 610e | 452e |
| 10.0 | 414f | 384f |

Means in each column followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to DMR test.

Table 6. Influence of selected fungicides at 2.5 kg ha$^{-1}$ on protease activity in black soils.

| Name of the Fungicide | 10 days | 20 days | 30 days | 40 days |
|---|---|---|---|---|
| Control | 422a | 384a | 553a | 224a |
| Mancozeb | 747b | 674b | 821b | 471b |
| Hexaconozole | 665c | 664b | 801b | 461b |

Means in each column followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to DMR test.

However, incubation of fungicide-treated samples up to 40 days resulted in no stimulation of the enzyme activity. Similarly as reported by Rasool and Reshi (2010), pesticide-induced (mancozeb) 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. The results in this study indicated clearly that the fungicides enhance the activities of dehydrogenase and protease in soil.

## Conclusion

Results obtained in the present study evidently specify that the fungicides, widely used in cultivation of cotton, at field application rates boost the activities of dehydrogenase and protease in black soil. The influence of fungicides on the enzyme activities increased with increasing concentrations of the fungicides up to 2.5 kg ha$^{-1}$ and 5.0 kg ha$^{-1}$ which was dose-dependent. Higher rates beyond 5.0 kg ha$^{-1}$ of the fungicides were either mild or toxic to the enzyme activities. The significant stimulation in the activity of dehydrogenase was more at 2.5 kg ha$^{-1}$, whereas the activity of protease was significantly increased at 5.0 kg ha$^{-1}$, lasted up to 21 days and 30 days respectively.

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***Cite this Article as:***

Murthy, V.K., Raffi, M.M. and Rangaswamy, V. 2022. Activities of dehydrogenase and protease in cotton cultivated black soils as influenced by fungicides. *J. Acad. Indus. Res.* 10(3): 50-54.

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