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# Impact of agrochemicals on the activities of soil amylase

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**Abstract**
Soil amylase is one of the crucial extra cellular enzymes produced by the soil microbes. It breaks down the starch into dextrin and smaller polymers composed of glucose molecules. Excess use of agrochemicals, affects the activity of this starch hydrolysis in a negative way. Though many researchers have observed the negative impact of pesticides on soil amylase, a brief review article provides a brief idea on soil amylase, its classification, its significance and its correlation with different pesticides.

**Keywords:** Soil amylase, pesticide, agrochemicals, chemical stressors

## Introduction
The primary function of soil enzymes is not only to measure the biological activity of the soil, but also, they response to several biological and biochemical changes in the soil profile due to environmental factors or external management like soil compaction, tillage, crop rotation, agrochemical applications etc. Soil enzymes are produced from living and dead microbes, plant roots and residues and soil animals. However, vegetation can also potentially affect soil enzyme content directly or indirectly as roots can excrete extracellular enzymes. These enzymes catalyse numerable reactions necessary for the sustenance of soil microbes, nutrient cycling, decomposition and formation of organic matter.

The enzyme amylase is essential to soil biological activity because it breaks down complex carbohydrates into simpler sugars, which promotes microbial development and nutrient cycling. It contributes to the breakdown of starch molecules into forms that are easier for microbes to access, promoting their metabolic activities and general activity in the soil environment (Singaram and Kamalakumari 2000) [16]. Because it affects the availability of nutrients for plants and other ecosystem species, amylase activity is crucial for the health of the soil. Monitoring amylase levels in soil can serve as an indicator of soil health and the impact of pesticides on soil enzyme activity, helping in the development of strategies to mitigate negative effects and promote sustainable soil management practices. However, a brief package of information related to the structure, significance and activity of soil amylase in relation to natural parameters and modern man-made actions are too scarce. Therefore, the authors have tried to focus on these specific area so as to make an eye-catching review on soil amylase.

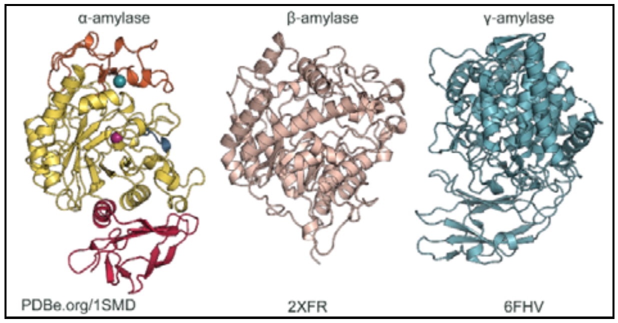
## Classification of Amylase
Amylases are group of enzymes which break down starch molecules into dextrin and other small glucose units by catalysing the hydrolysis of internal α-1,4-glycosidic bonds. As the reaction splits a molecule of water into $OH^-$ and $H^+$ ions which are bound to the exposed ends of the broken starch polymer; the reaction is named hydrolysis.

Based on the site of action, amylase enzymes are broadly classified as: α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and, γ-amylase (EC 3.2.1.3) (Tiwari *et al*., 2015) [21]. However, some authors have classified amylase as α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3) (Goesaert *et al*., 2009) [4]. α-and β-amylases are widespread in the soil as α-amylase is synthesized by plants, animals and microorganisms, while β-amylase is synthesized mainly by plants (Gunjal *et al*., 2019) [5].

α-amylase is an endo-amylase that can cleave α-1,4- glycosidic bond present in the inner part (endo-) of amylose or amylopectin chain. On the contrary, β-amylase is a well-known exo-amylase which cleaves α-1,4- glycosidic bond which is exclusively present on the external residue of amylose and amylopectin; thus, produces only one glucose at a time (Tiwari *et al*., 2015) [21].

Unlike the other two enzymes,γ-amylase is most efficient in acidic environment with an optimum Ph of 3. This enzyme has the capacity of cleaving α-1,6 glycosidic linkage, in addition to cleaving the external α-1,4 glycosidic bond from the non-reducing end of amylose or amylopectin (Tiwari *et al*., 2015) [21].

As per Goesaert *et al*., (2009) [4] β-amylases and glucoamylases are typical inverting exo amylases and hydrolyse the α-(1,4)-linkages at the non-reducing ends of starch molecules. β-amylase cannot hydrolyse or pass α-(1,6)-linkages; its action stops at the branch points. β amylase (potentially) degrades starch to b-maltose and b-limit dextrins. Glucoamylase has a limited activity on a-(1–6) bonds and, theoretically, can completely degrade starch to glucose.




**Source of image:** Protein Data Bank in Europe (ebi.ac.uk)

**Fig 1:** Molecular structure of α-amylase, β-amylase and γ-amylase.

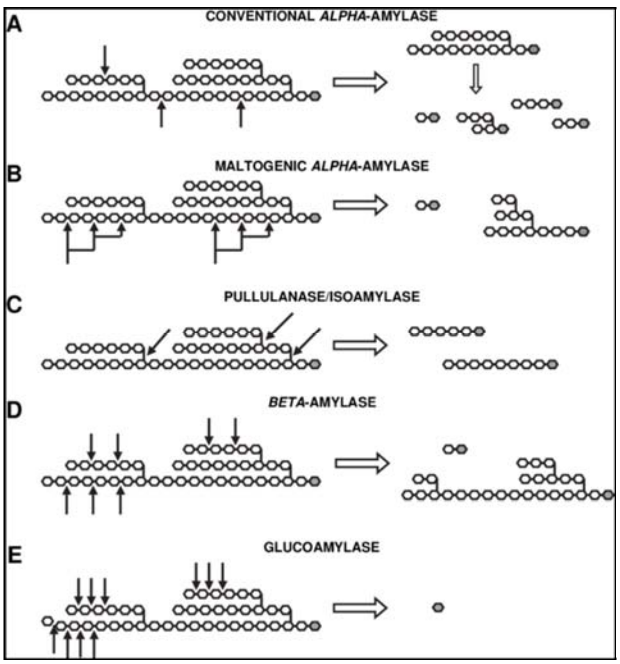



**Fig 2:** Action of different type of amylase on substrates (Goesaert *et al*., 2009) [4]

**Estimation of soil amylase**

The breakdown of substrates and the production of products serve as indicators of enzymatic activity. The techniques of radiolabelling, fluorescence, and spectrophotometry are employed to quantify enzyme activity (Gunjal *et al*., 2019) [5]. However, the technique of spectrophotometry has been used highly in case of soil amylase estimation.

Some authors have adopted the method of Somogyi M (1952) [18] to estimate the amylase activity in soil. 5 g of soil is added to 1.5 ml toluene and the mixture is gently shaken followed by 15 minutes of stable incubation after which, 10 ml distilled water and 5 ml of 2% soluble starch solution are added to it. The reaction mixture is incubated at 37 °C for 5 hours. Once the incubation is over, 15 ml distilled water will be added to the soil mixture followed by centrifugation at 3000 rpm. The absorbance of the clear supernatant is read at 600 nm using spectrophotometer. The enzyme activity is expressed as µg glucose released/gram oven dry soil/ hour at 37 °C.

As per the method prescribed by Ross & Robert (1970), 3 g of soil sample is incubated overnight at 37 °C with reaction mixture containing 0.2 mL of toluene, 6 mL of Sorenson's buffer (0.06 M) and 6 mL of substrate. Starch, the prospective substrate used for the assay of amylase enzyme. The supernatant is collected after centrifugation of the samples for 30 mins at 6000 g and 2 mL 3,5-dinitrosalicylic acid (DSA) is added to it. The readings are recorded at 540 nm with UV-Vis spectrophotometer. The enzyme activity is expressed as mg glucose/ug glucose /g VC /h.

**Sources of soil amylase**

Like other soil extracellular enzymes, various sources of soil amylase are plant, fungi and bacteria. However, most of the researchers of this field have focused on the microbial sources. Swaleha & Girish (2023) [20] isolated amylase-producing bacteria i.e *Bacillus cereus, Bacillus alvei, Bacillus licheniformis* from sorghum farm soil, highlighting their potential for enzymatic production under optimized conditions. Positive hydrolysis test was conducted by Gadhave *et al*. (2022) [3] and bacteria of *Basilus* genus was isolated as a significant producer of amylase. A study screened soil samples from Nyingchi, Tibet and identified 127 amylase-producing bacteria out of which 67.72% were confirmed to be of *Bacillus* genus by 16S r-RNA survey (Liu *et al*., 2022) [8]. Pan I (2021) [14] identified Bacillus subtilis B1U/1, a potent amylase-producing bacterium from compost soil and could observe its efficient ability of degrading bacterial biofilm.

**Impact of pesticides on the activities of soil amylase**

Amylase activity is essential for soil health as it influences the availability of nutrients for plants and other organisms in the ecosystem. Different pesticides applied to soil can impact amylase activity, affecting its ability to efficiently break down starch compounds, which may disrupt the soil microbial community and nutrient cycling processes. Tu CM (1988) [23] conducted a test with 39 pesticides, including 22 insecticides, 8 herbicides, 3 fungicides, 5 fumigants and a nitrification inhibiter and observed the activities of soil amylase. Tu CM (1990) [24] worked with four insecticides, D0WCO429X, DPX43898, Tefluthrin and Trimethacarb and noticed no significant changes in sandy soil amylase activities in early days but soon after stimulatory effects were observed in most of the samples. Amylase activity was reduced when soil was treated with imidacloprid (Tu C M 1995) [25], dimethoate, and chlorothalonil (Singh *et al*. 2002) [17].

When the soil was applied with a moderate dosage of Baythroid, an insecticide, higher activity of amylase was observed; whereas higher dosage of the insecticide reduced the activity level of soil amylase down to a greater level (Lodhi A, 2000) [9]. According to the research work of Ataikiru *et al*., (2019) [1], the impact of carbofuran and paraquat were observed on soil amylase activity separately. As per the result, the amylase activity reached to its peat by day 7 and then declined; but in case of paraquat, the amylase activity increased throughout the study period. Different insecticides like quinalphos, monocrotophus and cypermethrine put negative impact on amylase activity while use in soil separately or combinedly. Vani *et al*., (2012) [26] observed reduced activity of soil amylase at higher concentration of endosulfan and qunilphos. Methamidophos, an organophosphate at its higher concentration brought a marked reduction in the amylase activity throughout the entire study period (Ismail *et al*., 1999) [6].

While conducting an experiment with mancozeb and triadimefon, it was found that mancozeb (Walia *et al*., 2014) [27] and triadimefon (Deborah *et al*., 2013) [2] simulated soil amylase at low doses (mancozeb: 10 ppm and triadimefon: 0.2 kg/ha), while its activity declined at higher doses (mancozeb: 200 – 2000 ppm and triadimefon: 0.5 – 0.7 kg/ha). Triadimefon was much more toxic to soil amylase than mancozeb, since even a concentration of 0.5 kg/ha (6.8 mg/kg) inhibited the enzyme (Kenarova & Boteva, 2023) [7]. Separate application fungicides i.e Ridomil Gold and Bravo 500 and insecticides i.eMospilan 20SG and Vertimecled to significant reduction of amylase activity. Like other pesticides, higher concentration of endosulfan and profenofosbrought significant reduction in the amylase activity of cultivated black soil (Nasreen *et al*., 2012) [13]. In case of the use of Cartap hydrochloride, one of the main insecticides used in India, the amylase activity was found to be reduced during the early period of experiment followed by an increment. However, higher concentration of this insecticide became able to reduce the enzyme's activity. Increased amylase activity was observed when soil was treated with insecticides and pesticides (Tu C M, 1982) [22].

## Conclusion

Amylase activity is essential for soil health as it influences the availability of nutrients for plants and other organisms in the ecosystem. Different pesticides applied to soil can impact amylase activity, affecting its ability to efficiently break down starch compounds, which may disrupt the soil microbial community and nutrient cycling processes. Understanding the influence of pesticides on amylase activity is crucial for monitoring soil health and ensuring sustainable agricultural practices. Research on the interaction between pesticides and amylase can provide insights into maintaining soil biological activity and ecosystem functions in the face of chemical stressors.

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