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Effect of Pesticides on Enzyme Activities in Paddy (*Oryza sativa* L.) Soils

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Abstract

The activity of soil enzymes is often employed as a marker of soil contamination. In paddy (black and alluvial) soil, responses of specific soil enzymes, amylase, invertase, cellulase, and myrosinase, were studied at different concentrations (10, 25, 50, 75, and 100 ppm) of four pesticides, carbosulfan, chlorpyrifos (insecticides), and kresoxim methyl, mancozeb (fungicides), which are comparable to field treatment rates (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}). There was a significant rise in amylase, myrosinase activity at 2.5 kg ha^{-1}, and invertase and cellulase activity at 5.0 kg ha^{-1} in paddy (black and alluvial) soil after a 10-day incubation period. Furthermore, when pesticide concentrations increase to between 7.5 and 10.0 kg ha^{-1}, enzyme activity decreases. However, the stimulatory impact on four enzyme activities was maintained for 20 and 30 days, and the lowest enzyme activity was detected at 40-day incubation in paddy soil.

***Key words*:** Pesticides, Amylase, Myrosinase, Invertase, Cellulase, Paddy soils

Many interactions between living and non-living components are facilitated by the soil ecosystem. These interactions are crucial to the soil's biological activities and ecological functions [1-2]. Anthropogenic soil ecosystem disturbances, such as those produced by agriculture, mining, and other land use activities, have a negative effect on these vital connections [3-4]. Such soil quality monitoring methods will help determine the effectiveness of current reclamation operations and may provide information for future ecological function restoration. Various monitoring indicators for soil quality have been proposed and utilized on post-coal mining reclamation sites, including plant cover, erodibility, and compaction levels [5]. However, since the soil ecosystem is multidimensional in terms of biological, physical, and chemical components, these above-ground indicators do not offer a complete evaluation of soil health [6]. The applicability of soil biota as soil health indicators has gotten a lot of attention in recent years [7]. Because the richness and structure of the microbial population are susceptible to natural and human perturbations, the use of soil microbial indicators for soil quality monitoring has been suggested [8]. The direct relationship between soil microbial community diversity and soil ecosystem function [9], as well as the key roles of soil microorganisms in nutrient cycling, plant growth promotion, ecological succession, and energy flow in soil ecological food webs, support microbes' suitability as bioindicators [10-11]. For the reasons stated above, as well as to determine the potential impact of such disturbances on soil health processes and function, several studies have examined soil microbial community composition and function in anthropogenically and naturally disturbed environments [12-15]. Plant carbon (C) inputs are a significant source of energy for microorganisms, and they may be transformed into microbially generated C via microbial digestion and absorption [16-17]. Given the vast area (167 million hectares) covered by paddy fields worldwide, this is critical for raising soil C supply and moderating climate [18]. Because of its close contact and interactions with the soil mineral matrix, microbially generated carbon may be physically preserved in organ mineral associations, making it more stable than unprocessed plant-derived carbon [19]. Microbial decomposition products, including necromass and other byproducts, provide a substantial contribution to stable soil organic carbon (SOC) [20-21]. Plant residues can accumulate and persist in soils under anaerobic conditions, despite being less stable due to the lack of physical protection provided by minerals [22]. Cellulose is the most common organic molecule in the biosphere, accounting for almost half of all biomass produced by photosynthetic CO_2 fixation. As a consequence, detecting changes in the biological intensity process by monitoring enzyme activity in soil may be possible. Soil deterioration is caused by the interaction of physical and chemical properties of soil. Soil enzymes have an essential function in soil as a moderator and catalyst [23-24]. Because of industrial waste, agrochemicals, heavy metals, and soil fertility management that can be quantified, soil enzymology is now of practical importance [25]. Because of their relationship with soil ecology, sensitivity, operational practicality, simplicity of assessment, and integrative nature, enzymes in the soil are valuable biological indicators [23]. Enzymes are found in a variety of soil organisms, including non-living and living microbes, plant wastes and roots, and earth animals [26]. Many other bioindicators, such as plants and animals impacted by

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natural or human disturbance, are less reliable and sensitive than soil enzyme activity. They react rapidly to produced alterations. Enzyme activity, on the other hand, may be affected in a major or small way by unknown natural and human activities [23]. Accumulation of cellulose and polysaccharides of -1, four linked glucose units are degraded by cellulases [27]. Amylase is a soil enzyme that hydrolyzes polysaccharide or starch bonds to generate glucose and maltose [25]. The hydrolysis of sucrose to fructose and glucose is catalyzed by invertase [28].

MATERIALS AND METHODS

Soil samples were obtained from black and alluvial paddy farmed fields in the Proddatur and duvvur manadalam Kadapa districts, and were taken from a depth of 12 cm, air-dried at room temperature, sieved with a 2 mm sieve before use, and fully combined to create a homogenous composite sample.

Table l Physico-chemical characteristics of paddy soils

Properties	Black soil	Alluvial soil	Method
Sand (%)	50	57.4	
Silt (%)	22	25.7	
Clay (%)	28	16.9	
pH [a]	8.26	7.8	a = 1.25 (Soil : Water)
Water holding capacity (ml g^{-1} soil)	48.8	56	
Electrical conductivity (m. mhos)	0.19	0.31	
Organic matter (%) [b]	0.86	0.126	Walkley-Black technique [31]
Total nitrogen (%) [c]	0.54	0.80	Micro-Kjeldahl method [31]
NH_4^+–N(μg^{-1} soil) [d]	4.42	6.24	Nesslerization method [31]
NO_2^-–N(μg^{-1} soil) [e]	5.32	8.23	Diazotization method [32]

Analytical techniques for physicochemical property characterization of soil samples

According to Alexander's method [29], the contents of mineral matter in soil samples, such as sand, silt, and clay, were analyzed using different diameters of sieves. The percentage of water-holding capacity of soil samples was calculated by dividing the amount of distilled water given to both soil samples to achieve saturation point by 60% [30]. Soil pH was measured at a 1:1.25 soil to water ratio using a Systronics digital pH metre with calomel glass electrode assembly. The organic carbon content in soil samples was estimated using the Walkley and Black method, and the organic matter content was calculated by multiplying the findings by 1.72 [31]. The electrical conductivity of the soil samples was determined using the Conductivity Bridge after adding 100 mL of distilled water to 1 gramme of soil samples. The Micro-kjeldhal technique was used to determine the total nitrogen concentration in soil samples [31]. Following extraction with 1M KCl, the amount of inorganic ammonium-nitrogen in soil samples was measured using the Nesslerization method [31]. After extraction with water, the concentrations of nitrite-nitrogen [32] and nitrate-nitrogen [33] were determined using the Brucin technique [33]. (Table 1) shows the physical and chemical characteristics of paddy black and alluvial soil.

Insecticides utilized in this research

The effect of two different insecticides and fungicides on paddy soil enzyme activities was tested using Bayer scientific India market grades of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb (Table 2).

Table 2 The insecticides and fungicides used in this study were described in detail

Pesticides	Trade name	Commercial formulation	Chemical Class	Technical grade (% purity)	IUPAC
Carbosulfan	Marshal	25% EC*	Carbomate	99.8	2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl [(dibutylamino)sulfanyl] methylcarbamate
Cholrpyrifos	Noban	50% EC*	Orgnophosphate	97.54	O,O-diethyl O-(3,5,6-trichloropyridin-2-yl) thiophosphate
Kresoxim Methyl	Ergon	44.3% SC*	Strobilarin	94	methyl (αE)-α-(methoxyimino)-2-((2methylphenoxy) methyl) benzeneacetate
Mancozeb	Sparsh	75% WP*	Dithiocrbomate	95	zinc;manganese(2+);*N*-[2-(sulfidecarbo thioylamino) ethyl] carbamodithioate

EC* = Emulsifiable concentration, SC*= Suspension concentration, WP*= Wettable powder

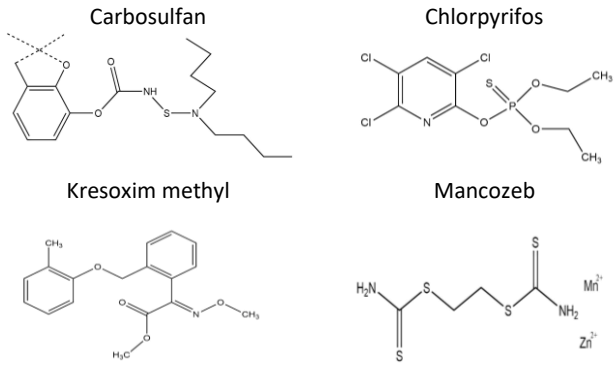


Fig 1 Pesticides chemical structure

Soil enzyme activities

Appropriate amounts of soil samples were deposited in test tubes or Erlenmeyer flasks and treated with various insecticide concentrations corresponding to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1} to determine the interaction effects of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb (10, 25, 50, 75, and 100 g g^{-1} soil) on the activities of soil enzymes. Soils that were not sprayed with pesticides were utilized as controls. All soil samples in test tubes or flasks were kept at room temperature (28°C) with a water holding capacity (WHC) of 60% during the incubation phase. At the appropriate intervals, samples were gathered for enzyme activity assessment (10, 20, 30, and 40 days).

Amylase assay

Cole [34] invented the amylase assay technique, which was followed by Tu [35-36]. To stop the enzyme activity, the soil samples were transported to 100 ml Erlenmeyer flasks and treated with 1 ml toluene. After 15 minutes, each of the test samples received 6 ml of 0.2M acetate phosphate buffer (pH 5.5) containing 2% starch and cotton plugs. The testing samples were made up to a volume of 50 ml with sterile distilled water and passed through Whattman No. 1 filter paper, and the filtrate was assayed for the amount of glucose by the Nelson method [37] followed by Jaffer Mohiddin *et al*. [38] in a U.V. Spectrophotometer after 24 and 72 hours of incubation (Thermo Scientific).

Invertase assay

The substrate sucrose (18 mM) was added to the soils and incubated for 24 and 48 hours to test invertase enzyme activity. The quantity of glucose released from sucrose was determined using the amylase activity technique.

Cellulase assay

Deng and Tabatabai's [39] method for measuring cellulase enzyme activity in soils has been used. In order to evaluate the amount of reducing sugar in the filtrate, 10 ml of carboxy methyl cellulose (CMC) 1 percent was employed as a substrate, followed by 10 ml of acetate buffer and incubation for 24 hours. After 10, 20, 30, and 40 days after incubation with pesticides, the enzyme activity may be measured.

Myrosinase Asaay

As previously stated, one-gram pieces of each soil were treated with the chosen pesticides in triplicates. After a 10-day incubation period, each tube was filled with 0.2 ml of toluene, 2.3 ml of N-tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES) buffer (0.1 M, pH 7) and 0.5 ml of sinigrin produced in 0.1 M TES buffer (pH 7), resulting in a final concentration of 20 mM. To mix the contents, the tubes were spun for a few seconds. The tubes were sealed and maintained at 37 degrees Celsius. The contents were placed into 50 ml plastic centrifuge tubes after 4 hours, and the soil suspension was centrifuged at 8000 g for 10 minutes before being filtered through a 0.45 m MF-millipore membrane filter into the test tube. 2 ml of reagents from the diagnostic glucose kit were pipetted into a labelled test tube and allowed to warm to assay temperature for the centrifugation time. By gently inverting, one ml of the filtered supernatant was added to the labelled test tube containing the 2 ml of reagents from the diagnostic kit. The tubes were incubated for precisely 20 minutes at room temperature (28 ± 4°C), after which 25 l of $AgNO_3$ (1 M) was added to halt all enzyme activity. A spectrophotometer set to a wavelength of 505 nm was used to measure the concentration of the pink colour of the outcomes complex [40].

RESULTS AND DISCUSSION

Amylase activity

Soils were treated with different concentrations of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb (1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1}) by exposing them to starch for 24 and 72 hours at room temperature (28 4°C) to evaluate the impact of pesticides on the activity of soil amylase enzyme and by incubating for ten days to evaluate the Carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb were treated in graduated doses of 1.0, 2.5, and 5.0 kg ha-1 in both soils, and a progressive rise in amylase activity was noted. After 10 days of incubation in black soil, carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 10 and 25 ppm levels caused increases in amylase activity of 12-40, 6-28, 18-62, 22-50 percent and 31-43, 26-173, 6-36, 13-38 percent beyond control, respectively (Table 3). At 10-day incubation, the comparable values of increase in amylase activity in alluvial soil with four pesticides at 10 and 25 ppm levels were 18-38, 14-25, 25-41, 19-27 and 10-33, 4-21, 30-55, 16-28 percent (Table 3). The findings showed that amylase activity was comparable in both soil and water (Table 3). After 72 hours of incubation with starch compared to 24 hours of incubation, the amylase activity in treated soil samples was greater than in untreated soil samples (Table 3). There was a significant increase in amylase activity in terms of glucose released from starch in both soils treated with 2.5 kg ha^{-1} of four pesticides.

Table 3 Pesticide activity on amylase activity in paddy black and alluvial soil after 24 hours and 74 hours after 10 days under the effect of various pesticide concentrations

Concentration	Carbosulfan		Chloropyrifos		Kresoxim-Methyl		Mancozeb	
of pesticides	24hrs	72hrs	24hrs	72 hrs	24hrs	72 hrs	24hrs	72hrs
				Black soil				
0.0	160f (100)	180e (100)	160e (100)	180e (100)	160e (100)	180e (100)	160e (100)	180e (100)
1.0	180c (112)	202d (106)	190d (118)	220d (122)	210c (131)	240b (126)	170d (106)	215c (113)
2.5	225a (140)	245a (128)	260a (162)	274a (150)	230a (143)	250a (273)	219a (136)	264a (138)
5.0	210b (131)	230b (121)	240b (150)	260b (136)	214b (133)	205c (107)	188b (117)	224b (117)
7.5	170d (106)	210c (110)	218c (136)	238c (125)	200d (125)	189d (99)	174c (108)	180d (94)
10.0	150f (93)	164f (86)	140f (87)	170f (94)	140f (87)	168f (88)	134f (83)	170f (89)
				Alluvial soil				
0.0	180e (100)	210e (100)	180e (100)	210e (100)	180f (100)	210e (100)	180f (100)	210e (100)
1.0	214c (118)	240c (114)	225c (125)	250b (119)	199d (110)	220c (104)	234d (130)	245c (116)
2.5	249a (138)	264a (125)	254a (141)	268a (127)	240a (133)	256a (121)	280a (155)	270a (128)
5.0	225b (125)	250b (119)	241b (133)	240c (114)	220b (112)	233b (110)	260b (114)	254b (120)
7.5	208d (115)	232d (110)	220d (133)	210d (100)	202c (112)	218d (103)	244c (135)	234d (114)
10.0	160f (88)	180f (85)	170 (94)	190f (90)	184e (102)	178f (87)	210e (116)	205f (97)

After 24 and 72 hours of incubation with 2% starch, µg soil was produced.

Figures with parenthesis represent percentages of comparative productivity.

According to the DMR test, the means obtained for each sample in each row, followed by the same letter, are not statistically different ($P \leq 0.05$) from each other.

The values in the table are the averages of three replicates.

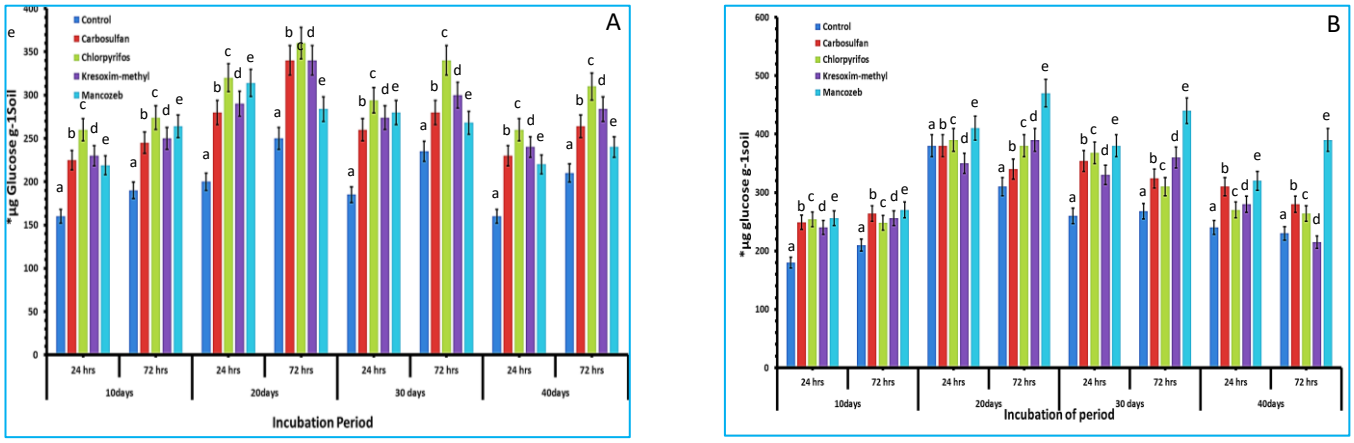


Fig a-b Pesticide effects on amylase* in 2.5 kg ha^{-1} paddy black and alluvial soil

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test. Bars in the figures represent means of three replicates.

*Values plotted in figure are means of triplicates.

Glucose deposition was raised in all treated and untreated soils after 20 days of incubation, although it gradually reduced with additional incubation (Fig a-b). The elevation in enzyme activity was also greatly increased whenever the substrate was applied to the samples taken for 72 hours.

The sprayed pesticides increased amylase activity more at 2.5/5.0 kg/ha, while higher doses (7.5 to 10.0 kg/ha) were still extremely toxic to soil amylase activity in the present study. Vijaya *et al*. [41] found that increasing pesticide doses were either harmful or benign to amylase activity. At high doses, triadimefon decreased the incidence of amylase enzyme activity. At 50 mg/L and 10 mg/L, the effects of two triazole drugs, triadimefon and hexaconazole, on carbohydrate metabolism were investigated. These triazoles resulted in a slight increase in starch content while lowering sugar levels. The activities of and amylase were inhibited when triadimefon and hexaconazole were used [42]. Similarly, many studies have found an increase in amylase activity following pesticide treatment [43-44]. Walia [45] explored the role of fungicides on soil enzymes, finding that mancozeb doses of 10 to 2000 mg kg^{-1} inhibited amylase activity. Vijaya Vani *et al*. [46] found similar results in paddy soils using endosulfan and quinalphos. In this research, the enzyme activity of the black soil was greater than the alluvial soil.

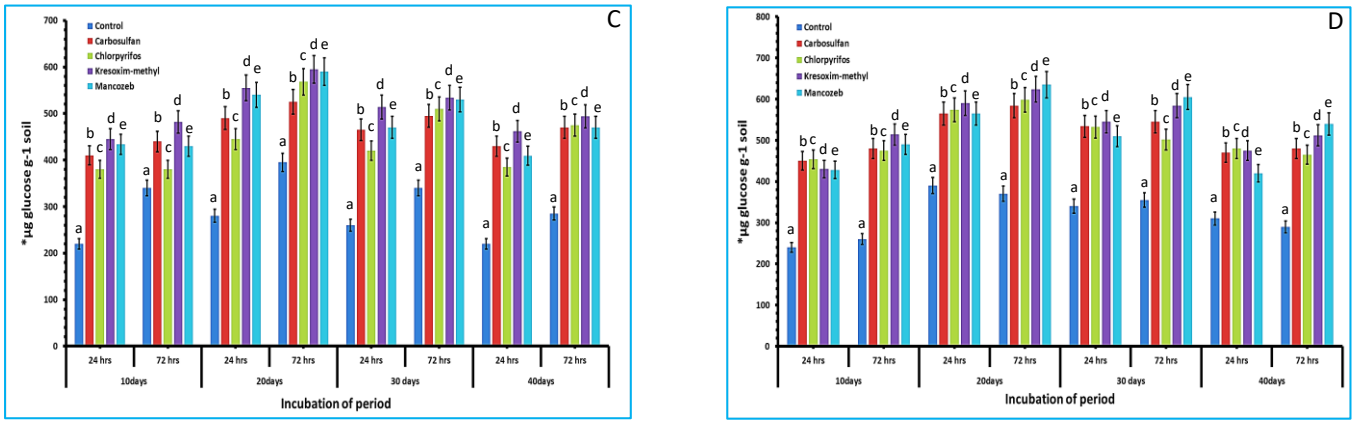


Fig c-d At 5.0 kg ha-1, the effect of pesticides on invertase* in a paddy black and alluvial soil

According to the DMR test, the means obtained for each sample in each row, followed by the same letter, are not statistically different ($P \leq 0.05$) from each other.

The values in the table are the averages of three replicates.

Invertase

To evaluate the impact of pesticides on the activity of the soil enzyme invertase, soil samples were treated with various doses of (1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1}) of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb and incubated for 10 days (Tables 4). Invertase enzyme activity was evaluated at 24 and 48 hours in soils treated for 10 days under the effect of different pesticides, and expressed as the amount of glucose generated from the source, sucrose. Both black and alluvial soil samples treated with 2.5 kg ha^{-1} of carbosulfan, chlropyrifos, kresoxim-methyl, and mancozeb produced substantially more glucose from sucrose. Pesticides (Carbosulfan, chlropyrifos, kresoxim-methyl, and mancozeb) at 10 and 25 ppm levels induce increases in invertase activity of 27-86, 15-29, 18-72, 17-41 percent and 22-102, 14-41, 35-97, 13-41 percentages in black soil for 24 and 48 hours beyond control, correspondingly, at 10-day intervals (Table 4). Similarly, at 10, 25 ppm, the four pesticides caused substantial increases in invertase activity of 16-87, 23-84, 18-89, 19-82 percent and 12-59, 28-97, 20-78, 19-88 percent above control, respectively, in red soil for 24 and 48 hours (Table 4). The (Table 4) shows the activity of invertase during 10 days of exposure to different pesticide doses. When these pesticides were treated at 7.5 and 10.0 kg ha-1, the generation of glucose from sucrose was substantially reduced. The enzyme activity increased more in soils treated for 20 days with substrate at 48 hours, then decreased as the incubation time increased (Fig c-d). All of the treated soil samples had increased determined enzyme activity when compared to the 20-day measured soil samples and the 30 and 40-day incubated soil samples (Fig c-d).

The consequences were not strong or lengthy enough to be considered detrimental to invertase activity, which is essential for soil fertility [47]. Arinze and Yubedee [48], on the other hand, found that perhaps the kitten and otherwise fenvalerate suppressed enzyme activity. Invertase activity was greater in black soil at a concentration of 5.0 kg ha^{-1}. Individual applications of fungicides, chlorothalonil, and propiconazole at 1.0, 2.5, and 5.0 kg ha^{-1} substantially enhanced invertase activity in both black and red soils [49].

Cellulase activity

The enzyme cellulase catalyses the conversion of cellulose to glucose. The effect of different pesticides on cellulase activity was assessed due to the enzyme's critical role. In both soils, cellulase expansionary activity was greatly increased at 5.0 kg ha^{-1}. Large amounts of sprayed pesticides (7.5 and 10.0 kg ha^{-1}) showed either a decreasing or benign effect on cellulase activity across both black as well as alluvial soil samples (Table 4). Pesticides carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb increased cellulase enzyme activity in black and red soils by 30-112, 33-92, 23-75, and 37-99, respectively, beyond control in the black soil sample at 5.0 kg ha^{-1} during a 10-day incubation period (Table 5). Cellulase activity increases 15-75, 23-64, 28-73, and 32-82 in alluvial soil samples at 5.0 kg ha^{-1}, accordingly (Table 5). Cellulase activity is greater in black soils than it is in alluvial soils, according to a recent study. The pesticides had an effect on cellulase activity throughout the incubation period, both separately and in combination. At day 30 of incubation, there was a higher level of cellulase activity. Cellulase activity was either boosted or decreased as the incubation time increased up to 40 days (Fig e-f).

Table 4 Pesticide activity on invertase activity in paddy black and alluvial soil after 24 hours and 74 hours after 10 days in the presence of various pesticide concentrations

Concentration	Carbosulfan		Chloropyrifos		Kresoxim-Methyl		Mancozeb	
of pesticides	24hrs	72hrs	24hrs	72 hrs	24hrs	72 hrs	24hrs	72hrs
				Black soil				
0.0	220f (100)	340f (100)	220f (100)	340e (100)	220f (100)	340f (100)	220f (100)	340e (100)
1.0	280d (127)	394e (115)	260e (118)	380d (117)	270e (122)	390d (114)	299d (135)	385c (113)
2.5	340c (154)	420b (123)	297c (135)	425b (125)	338c (153)	454b (133)	390c (177)	430b (126)
5.0	410a (186)	440a (129)	380a (172)	480a (141)	445a (202)	482a (141)	434a (197)	480a (141)
7.5	380b (172)	400c (117)	345b (156)	424c (124)	405b (184)	448c (131)	395b (179)	360d (105)
10.0	270e (122)	360d (105)	288d (130)	320f (94)	310d (140)	370e (108)	240e (109)	320f (74)
				Alluvial soil				
0.0	240f (100)	260f (100)	240f (100)	260f (100)	240f (100)	260f (100)	240f (100)	260f (100)
1.0	280d (116)	320d (123)	285e (118)	310e (119)	270e (112)	335e (128)	290e (120)	370d (119)
2.5	340c (141)	380b (146)	376c (156)	398b (153)	355c (147)	482b (185)	345c (143)	440b (169)
5.0	450a (187)	480a (184)	454a (189)	475a (182)	430a (159)	514a (197)	428a (178)	490a (188)
7.5	380b (158)	374c (143)	410b (170)	424c (163)	405b (168)	470c (180)	380b (158)	364c (140)
10.0	270e (112)	280e (107)	320d (133)	348d (133)	340d (141)	364d (140)	310d (129)	280e (107)

*µg glucose g^{-1} soil formed after 24 and 48 hrs incubation with 18mM sucrose.
Figures with parenthesis represent percentages of comparative productivity.
According to the DMR test, the means obtained for each sample in each row, followed by the same letter, are not statistically different ($P \leq 0.05$) from each other.
The values in the table are the averages of three replicates.

Table 5 Cellulase* activity in paddy soils for 24 hours after 10 days there under the influence of various pesticide doses

Pesticide concentration (kg ha^{-1})	Carbosulfan	Chloropyrifos	Kresoxim-methyl	Mancozeb
		Black soil		
0.0	280f (100)	280f (100)	280f (100)	280f (100)
1.0	365d (130)	375d (133)	345d (123)	385e (137)
2.5	474b (169)	440c (157)	410b (146)	477c (170)
5.0	595a (212)	540a (192)	490a (175)	559a (199)
7.5	430c (153)	470b (167)	395c (141)	501b (178)
10.0	310e (110)	330e (117)	301e (107)	425d (151)
		Alluvial soil		
0.0	320f (100)	320f (100)	320f (100)	320f (100)
1.0	370e (115)	395d (123)	410d (128)	425d (132)
2.5	485c (151)	440c (137)	453c (141)	510b (159)
5.0	560a (175)	525a (164)	555a (173)	584a (182)
7.5	520b (162)	496b (155)	468b (146)	504c (157)
10.0	430d (134)	348e (106)	330e (103)	405e (126)

*µg glucose g^{-1} soil formed after 24 hrs incubation with 1% carboxyl methyl cellulose (CMC).
Figures with parenthesis represent percentages of comparative productivity.
According to the DMR test, the means obtained for each sample in each row, followed by the same letter, are not statistically different ($P \leq 0.05$) from each other.
The values in the table are the averages of three replicates.

Cellulases were activated much higher in black soil than in red soil under the effect of pesticides, as per Srinivasulu and Rangaswamy [43]. Pesticide poisoning to soil microorganisms may be the cause of the enzyme's deficiency [50]. Pesticides had a higher stimulatory effect in black soil than those in alluvial soil, as per the current study's experimental findings. Jaffer Mohiddin *et al*. [38] discovered that cellulase activity in terms of glucose released as well as from cellulose was more evident at 0.5 kg/ha soil when both insecticides, imidacloprid and acephate, were used. At higher concentrations of 7.5 and 10 kg/ha, both insecticides were either stimulatory or benign to cellulase activity.

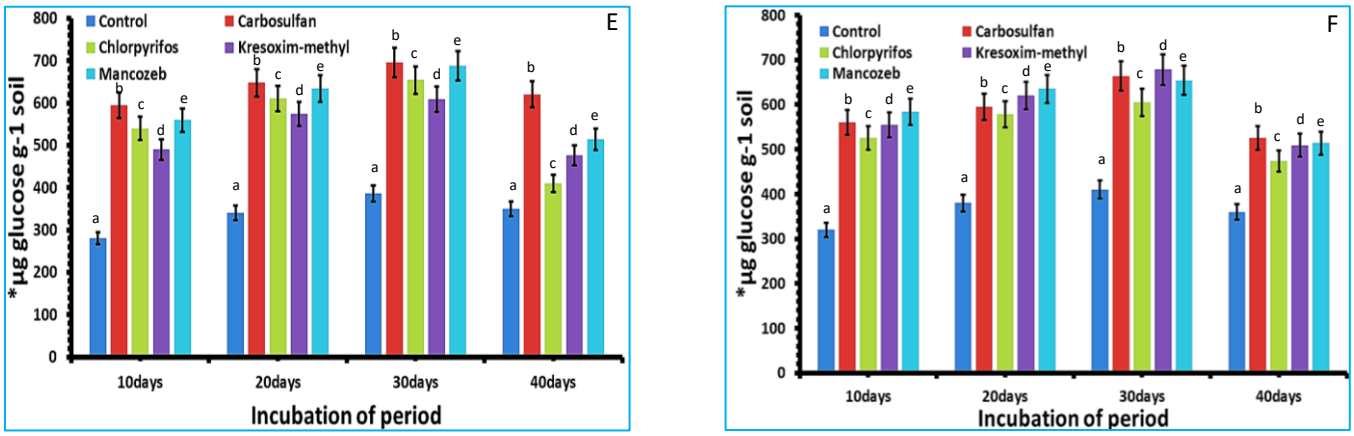


Fig e-f Effect of pesticides at 5.0 kg ha-1 on cellulase*activity in paddy soils, respectively.
Figures with parenthesis represent percentages of comparative productivity.
According to the DMR test, the means obtained for each sample in each row, followed by the same letter, are not statistically different ($P \leq 0.05$) from each other.
The values in the table are the averages of three replicates.

Cellulase activity in treated soil with two fungicides, brominal and selecron, was reduced at field technical efficiency and fivefold field rates after most incubation periods [51]. As per Moharram *et al*. [52], cellulase production was substantially decreased, especially at higher levels (200-400 ppm).

Myrosinase activity

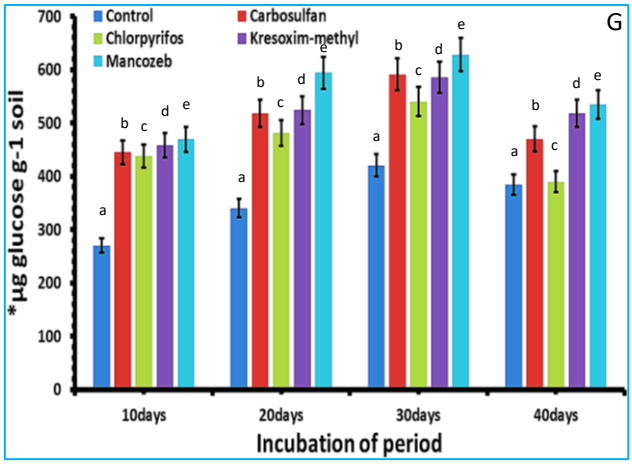
Myrosinase is indeed an enzyme involved in glucosinolate conversion to D-glucose and substances that have biological inhibitory potential for weed seeds. After 40 days of incubation, myrosinase levels progressively dropped (Fig g-h). Carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb increased myrosinase activity in soil at dosages ranging from 1.0 to 2.5 kg ha^{-1}. Myrosinase activity rose up to 7.5 kg ha^{-1} in all insecticide-treated soils in 10-day incubated soil samples compared to controls. With a value of 5.0 kg ha^{-1}, the activity of the enzyme was greatest in both soil samples. At 10.0 kg ha^{-1}, carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb all had little enzyme activity and had a detrimental effect (Table 6). When compared to control, myrosinase activity increased 40-64, 51-62, 55-69, and 40-73 percent in black soil and 13-38, 24-62, 35-70, and 26-40 percent in alluvial soil treated with carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb after a 10-day incubation (Table 6).

At 1.0 and 5.0 kg ha^{-1} of each pesticide, Srinivasulu and Rangaswamy [53] discovered that employing a combo of monocrotophos with mancozeb and chlorpyrifos with carbendazim enhanced myrosinase activity in black and red soils. However, greater pesticide dosages (10 kg ha^{-1}) had a detrimental impact on myrosinase activity, indicating that the two are antagonistic.

Table 6 After 10 days, the activity of myrosinase* in paddy soils was measured for 24 hours then under the influence of various pesticide doses

Pesticide concentration (kg ha^{-1})	Carbosulfan	Chloropyrifos	Kresoxim-methyl	Mancozeb
		Black soil		
0.0	270f (100)	270e (100)	270f (100)	270f (100)
1.0	380b (140)	410b (151)	420c (155)	380c (140)
2.5	445a (164)	438a (162)	458a (169)	469a (173)
5.0	370c (137)	400c (148)	402d (148)	418b (154)
7.5	316d (117)	356d (131)	454b (131)	374d (138)
10.0	286e (105)	268f (99)	300e (111)	298e (110)
		Alluvial soil		
0.0	340e (100)	340e (100)	340e (100)	340f (100)
1.0	385c (113)	424d (124)	459d (135)	430c (126)
2.5	470a (138)	551a (162)	578a (170)	478a (140)
5.0	450b (132)	503b (147)	501b (147)	440b (129)
7.5	360d (105)	470c (138)	480c (141)	410d (120)
10.0	330f (97)	335f (98)	310f (91)	350e (102)

*µg of glucose g^{-1} soil formed after 4 hours incubation with sinigrin. Figures with parenthesis represent percentages of comparative productivity. According to the DMR test, the means obtained for each sample in each row, followed by the same letter, are not statistically different ($P \leq 0.05$) from each other.
The values in the table are the averages of three replicates.



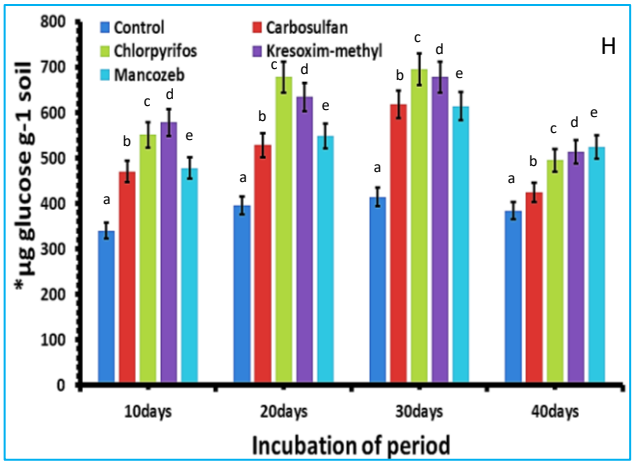


Fig g-h Pesticide effects on myrosinase* activity in a) black soil and b) alluvial soil at 2.5 kg ha^{-1}.
Figures with parenthesis represent percentages of comparative productivity.
According to the DMR test, the means obtained for each sample in each row, followed by the same letter, are not statistically different ($P \leq 0.05$) from each other.
The values in the table are the averages of three replicates.

CONCLUSION

The results indicate that over a 10-day period in paddy black and alluvial soils, amylase activity, represented in measures of glucose released from starch in soil, was greater at 2.5 kg ha^{-1} of four pesticide treatments in paddy black and alluvial soils. Regardless of the duration of substrate exposure, the toxicity of the insecticide treatment at higher concentrations (10 kg ha^{-1}) significantly decreased soil enzyme activity (24 hours and 72 hours). The enzyme activity was more apparent when the substrates were treated with soil samples for 72 hours. Insecticide-treated soils, on the other hand, had substantially greater enzyme activity than control soil samples. Invertase activity in treated and untreated paddy black and alluvial soil samples after 24 and 48 hours of incubation with substrate showed a similar trend in sucrose production. In rice soils, a pesticide initial treatment of 5.0 kg ha^{-1} substantially enhanced invertase activity. In both treated and untreated soils, invertase activity peaked at a 20-day interval and then decreased with additional incubation, comparable to amylase activity. At 5.0 kg ha^{-1} of the two pesticide treatments, the amount of glucose delivered by carboxymethyl cellulose (CMC) during cellulase activity was significantly enhanced both in paddy soil samples. Cellulase activity did not follow the same pattern as amylase and invertase activity. Four pesticides, like enzymes, resulted in a significant increase in myrosinase activity in both paddy soils at 5.0 kg ha^{-1}. Up to 30 days of incubation, the activity of myrosinase, measured in terms of glucose released as well from sinigrin in pesticide-treated soil samples, progressively rises. The findings of this research showed that applying lower dosages of pesticides to the rice crop (1.0, 2.5/5.0 kg ha^{-1}) may not have a negative impact on soil enzyme activities such as amylase, invertase, cellulase, and myrosinase, which are essential in soil nutrient cycling.

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