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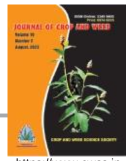

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Assessment of resistance and resilience of fluorescence in diacetate hydrolase activity under rice-wheat cropping system in an *Inceptisol*

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ABSTRACT

Concerned about the possible consequences of long-term climate change on resistance and resilience soil, this study was undertaken on a 11 years old long-term rice-wheat-mungbean cropping system based on conservation agriculture (CA) from ICAR-IARI, New Delhi. Collection of soil samples were done from 0-15cm depth and Fluorescein diacetate (FDA) hydrolase activity following heat stress of 48°C for 24 hours and 3 days of air drying were examined in the soil samples on day 1,15,30 and 45 days after stress (DAS). FDA activity varied from 16.9 ìg fluorescein g^{-1} soil h^{-} to 33.2 ìg fluorescein g^{-1} soil h^{-1}. Activity of FDA was reduced significantly by heat and moisture stress. The recovery rate of enzyme activity ranged from 61% to 83% at 45 DAS. The triple zero tillage treatment with triple residue retention demonstrated the highest resilience and resistance to heat and moisture stress among all the zero tilled treatments. Resistance and resilience index had a strong agreement with equivalent rice yield. In the Indo-Gangetic plain, where rice and wheat are grown in a CA-based system, triple zero tillage with triple residue retention may be advised.

Keywords: Conservation agriculture, FDA hydrolase activity, Inceptisol, resilience, resistance, stress

Prolonged droughts and increased temperatures are just a few examples of extreme climate events that significantly alter the way ecosystems are built and function. In most cases, these disturbances also prepare the way for unanticipated shifts from one ecological state to another. Additionally, due to human-induced climate change and anticipated rise in the frequency and magnitude of climate extremes, the possibility for such rapid changes in ecosystem equilibria is rising (Bardgett and Caruso, 2020; Bhowmik, 2014). A system's stability affects its capacity to keep operating in the face of changing circumstances, such as those brought on by either natural or anthropogenic disturbances. "Ecological stability consists of two components: resistance (the amount of change caused by a disturbance), and resilience (the speed with which a system returns to its pre-disturbance level following a disturbance)" (Pimm, 1984). According to certain theories, the makeup of the microbial community and the characteristics of the residing microorganisms could influence resistance and resilience (Griffiths *et al.*, 2013).

Soil enzymes are crucial to ecosystem functions due to the fact that they mediate a number of crucial reactions in biogeochemical cycles (Fanin *et al.*, 2022). A sensitive and non-specific test called fluorescein diacetate (FDA) hydrolysis can indicate the hydrolytic ability of soil microorganisms in a variety of soils (Dutta *et al.*, 2010). Enzymes associated with the microbial degradation of organic compounds in soil, such as esterases, proteases, and lipases, hydrolyze FDA (Gajda *et al.*, 2013). The FDA hydrolysis assay can estimate the total microbial activity in a soil sample by measuring the enzymatic activity of microbial communities (Szabo *et al.*, 2022). Widespread usage of the fluorescein diacetate (FDA) technique is done to quickly and accurately measure microbial activity. In the field of soil biological studies, FDA hydrolytic rate estimation has been frequently employed as a low-cost, simple and widely accepted method (Zhang *et al.*, 2022). The functional integrity of soil enzymes determines the longevity of soil biochemical activities, which is impacted by a variety of environmental stresses (Wang *et al.*, 2019). It is unclear how soil enzyme activities will change as a result of global warming. Therefore, great effort should be put into comprehending how soil ecosystems react to disturbance or environmental change, as well as how resilient and resistant soil microorganisms are.We hypothesized was that the resilience and resistance of FDA hydrolase activity would be significantly impacted by long-term conservation agriculture practices such as zero tillage, crop residue incorporation, green manuring,

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or in combination with synthetic fertilizers.Keeping all these things in mind, following research work has been done with the following objectives:1. To study the long-term effect of conservation agricultural practices on resistance and resilience of FDA hydrolase activity against heat and moisture stress and 2. To develop relationship between resistance and resilience of FDA hydrolase activity with yield as management goal.

MATERIALS AND METHODS

The experiment was conducted in the ICAR-Indian Agricultural Research Institute (IARI) Research Farm in New Delhi (28º35´N, 77º12´E). The climate in New Delhi is subtropical and semiarid, with cold winters and hot dry summers. 80% of the total amount of precipitation occurs from July to September and the remaining 20% occurs during "western disturbances" from December to February. The soil (order Inceptisol, Typic Haplustept) had a sandy loam texture, a pH of 7.8, an organic carbon content of 0.51%, an electrical conductivity of 0.64 dS m^{-1}, and amounts of available N, P, and K of 272, 10.2, and 208 kg ha^{-1}, respectively.The split plot design used for this study included eight main plot treatments and three replications.The treatments include triple and double zero tillage (ZT) without and with rice (RR), wheat (WR) and mungbean residues (MR) and also with Crotolaria (CBM) and Sesbania brown manuring (SBM). The detail description of treatments are given in Table 1.

After harvesting of wet season rice, Arize 6129 Gold on 30th October 2021, samples of soil were taken at a depth of 0 to 15 cm. During the soil sampling and sample preparation process, visible pieces of crop residues and gravels were removed. Each soil samples were divided into three parts, one part was stored in refrigerator at 4ºC to study biological parameters, remaining portion was dried and sieved for analysis of chemical and physical parameters.

Soil samples were provided with heat stress@ 48°±2 and 72 hours of air drying. Heat stress was provided in an incubator by placing soil samples inside glass beakers and covering with parafilm to check moisture loss. After providing stress, moisture content was maintained at one-third of field capacity. Incubation was done at 28°±2 for 45 days. Interval of study was 1,15,30 and 45 DAS. The method based on the determination of fluorescein released after the incubation of soil with fluorescein diacetate substrate for 3 hours at 37^0C was used to assess FDA hydrolase activity. The released fluorescein was extracted with acetone and the intensity of fluorescein was measured at 490 nm wavelength using a spectrophotometer. The enzyme activity is expressed as microgram of fluorescein formed per gram of oven dry soil per hour.

Resistance and resilience index were be determined (given below) as described by Orwin and Wardle (2004).

The index for resistance (RS)

$$RS\ (t_0) = 1 - \frac{2|D_0|}{(C_0) + |D_0|} \quad (1)$$

Where D_o refers to the difference between the control (C_0) and the disturbed soil (P_0) at the end of disturbance (t_0).

The index for resilience (RL) is

$$RL\ at(t_x) = \frac{2|D_0|}{(|D_0| + |D_x|)} - 1 \quad (2)$$

Where D_x refers to the difference between the control (C_x) and the disturbed soil (P_x) at the time point chosen to measure resilience (t_x).

To segregate the difference between mean values, the soil analysis data from the field experiment were statistically analyzed using Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984),using SPSS software (21.0).

RESULTS AND DISCUSSION

Fluorescein diacetate activity (FDA) of soil during sampling varied from 16.9 ìg fluorescein g^{-1} soil h^{-1} in conventional treatment (CT) to 33.2 ìg fluorescein g^{-1} soil h^{-1}in triple ZT with triple residue retention treatment (Table 2). Highest value was associated with triple ZT with residue treatment which showed the significant effect of zero tillage and retention of residue. The amount of enzymes that contribute to breakdown of soil organic matter (SOM) is measured by FDA hydrolysis. Greater soil physical conditions, or better protection for SOM, are offered by CA-based systems due to improved aggregation (Bhattacharyya *et al.*, 2019). The soil organic carbon (SOC) may have been stabilized by the use of ZT, however in CT plot, the SOC is not stable despite comparable yearly crop residue intake. A variety of soil microorganisms may have been able to thrive due to the surface soils' better hydro-thermal conditions, MWD, and well-aggregated soil structure under ZT (Dey *et al.*, 2016). Because of intense soil mixing during tillage practices under CT plots, there is relatively less undecomposed crop residue available on soil surface, which lowers the soil biological activities.

Heat stress lead to significant reduction in enzyme activity in all the treatments. The thermal degradation of enzyme protein and the catabolic activities of extracellular proteases may be accountable for the decrease in FDA activity following heat stress (Alvarez *et al.*, 2018). The decline in enzyme activity may have been caused by a decrease in enzyme secretion and synthesis in response to heat (Allison *et al.*,

Table 1 : Details of the treatments of long-term CA based rice-wheat cropping system, ICAR-IARI, New Delhi

SL No.	Treatment details
T-1	Zero till direct seeded rice (ZTDSR) – zero till wheat (ZTW)
T-2	Zero till direct seeded rice (ZTDSR) + wheat residue (WR) - zero till wheat (ZTW) + rice residue (RR)
T-3	Zero till direct seeded rice (ZTDSR) + wheat residue (WR) + crotolaria brown manuring (CBM) – zero till wheat (ZTW) + rice residue (RR)
T-4	Zero till direct seeded rice (ZTDSR) + wheat residue (WR) + sesbania brown manuring (SBM) – zero till wheat (ZTW) + rice residue (RR)
T-5	Zero till direct seeded rice (ZTDSR) – zero till wheat (ZTW) – zero till mungbean (ZTMB)
T-6	Zero till direct seeded rice (ZTDSR) + mungbean residue (MR) – zero till wheat (ZTW) + rice residue (RR) - zero till mungbean (ZTMB) + wheat residue (WR)
T-7	Transplanted puddled rice (TPR)- zero till wheat (ZTW)- zero till mungbean (ZTMB)
T-8	Transplanted puddled rice (TPR)-conventional till wheat (CTW) – conventionally tilled mungbean (CTMB)

Table 2: Effect of heat and moisture stress on FDA hydrolase activity (ìg FL g^{-1} soil h^{-1}) of soil under CA based R-W system

Treatments	0 DAS	1 DAS (H)	1 DAS (M)	15 DAS (H)	5 DAS 1(M)	30 DAS (H)	30 DAS (M)	45 DAS (H)	45 DAS (M)
T_1	23.3bc	15.4bcd	13.8cd	18.4bcd	17.6bcd	19.6bc	20.3bc	21.8bcd	21.2bcd
T_2	24.4bc	16.2bcd	14.6bc	20.9bc	18.7bc	21.1bc	18.7cd	22.9bc	22.3bc
T_3	22.8bc	17.5bc	16.4bc	20.7bc	18.6bc	21.8b	17.6cd	22.1bcd	21.9bc
T_4	25.2bc	19.9b	18.5b	21.6bc	22.0bc	22.7b	23.3bc	24.6bc	24.5bc
T_5	27.8b	21.7ab	18.8b	25.8ab	22.9b	25.1b	24.7b	26.8b	27.0ab
T_6	33.2a	26.8a	26.6a	29.9a	30.5a	31.6a	31.5a	32.6a	32.7a
T_7	20.4cd	12.8cd	12.5cd	17.7cd	16.2cd	18.2bc	17.8cd	19.2cd	18.6cd
T_8	16.9d	10.6d	9.59d	12.9d	12.6d	14.3c	14.2d	15.5d	15.1d

DAS, Days after stress; H, heat stress ; M, moisture stress; ±Values (mean) in each column (between the treatments) followed by different lower-case letters are significant according to Duncan's Multiple Range Test at P = 0.05

2018).Highest reduction (37%) was found in conventional treatments followed by $T\text{-}_1>T\text{-}_2>T\text{-}_3>T_{\text{-}5}>T\text{-}_4>T\text{-}_6$. Values of resistance index indicated that between the treatments, triple zero tillage with triple residue retention was most resistant whereas partly conventional and fully conventional treatments were least resistant to heat stress (Fig. 1). From the data on resilience indices it was observed that recovery was lowest in fully conventional treatment. Recovery upto 83% was found in triple ZT with residue at 45 days after stress (Fig. 2). The action of protease, lipase, and esterase contribute to FDA hydrolysis in soils (Green *et al.,* 2006). A possible explanation for the zero tilled soil's superior resistance to FDA hydrolysis activity is the "broad-scale" group of enzymes that express this kind of hydrolytic activity (Wang *et al.,* 2019). SOM have significant effects on the ability of soil enzymes to withstand heat stress. FDA hydrolase is known to form complexes with organic compounds and clay minerals present in the soil that can stabilise and prevent them from deteriorating so they can be active for longer periods of time (Wang *et al.,* 2019). The development of complexes between organic clay and enzyme and the defence mechanisms of organic compounds are reflected in heat stability. The survival or expansion of microbial populations will determine how resilient an enzyme is which may synthesize new enzymes, even though interactions between soil colloids and enzymes may affect how resistant an enzyme is to heat shocks (Chaer *et al*., 2009).

Effect of moisture stress was significant in all the treatments at 1 day after stress. Lowest FDA activity was found in fully conventional treatment and highest activity was reported in triple ZT with triple residue retention treatment whose values were 9.59 ìg fluorescein g^{-1} soil h^{-1} and 26.6 ìg fluorescein g^{-1} soil h^{-1}, respectively. Reduction in FDA was 43% in fully conventional treatment without residue and 20% in triple ZT with residue (Table 2). Value of resistance index indicated that triple ZT with triple residue retention was most resistant to moisture stress and fully conventional treatment was least resistant with RS value of 0.40 (Fig .1). As per the data on resilience index, recovery was upto 85% in triple ZT treatment with residue which

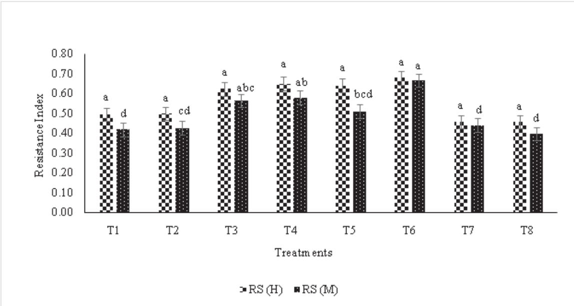

Fig. 1: Resistance of FDA hydrolase activity after heat and moisture stress of soil under CA based R-W system; RS, Resistance index; H, Heat stress; M, Moisture stress; ±Values (mean) in each bar (between the treatments) followed by different lower-case letters are significant according to Duncan's Multiple Range Test at P = 0.05

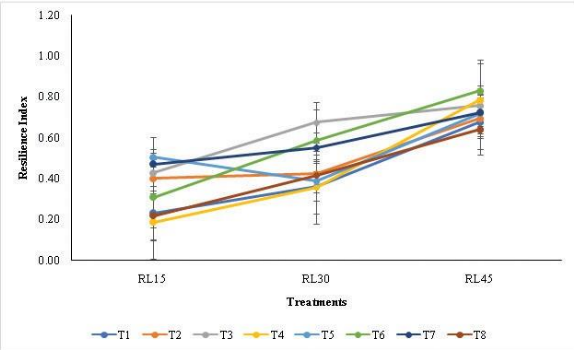

Fig. 2: Resilience of FDA hydrolase activity after heat stress of soil under CA based R-W system RL 15, 30, 45, resilience index on 15, 30, 45 days after stress respectively

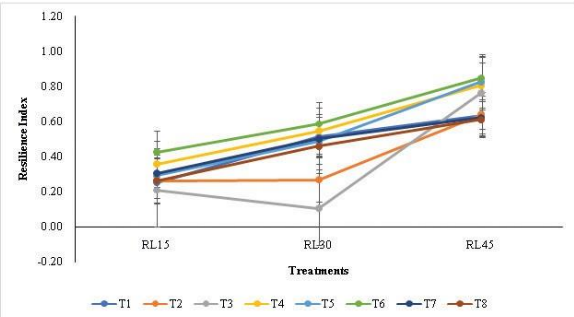

Fig. 3: Resilience of FDA hydrolase activity after moisture stress of soil under CA based R-W system RL 15,30,45, resilience index 15,30,45 days after stress respectively

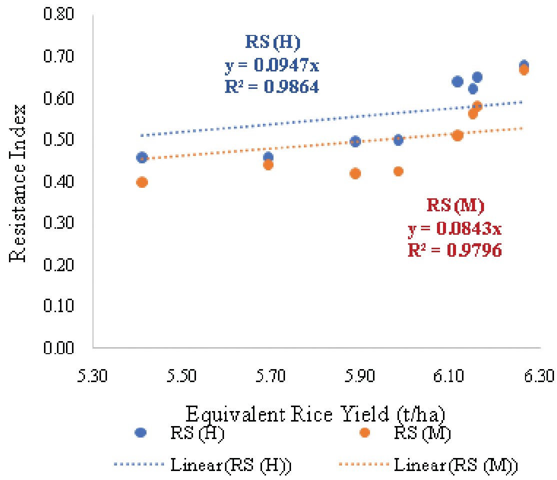

Fig. 4: Relationship between Resistance Index and Equivalent rice yield of soil under CA based R-W system RS, Resistance index; H, Heat stress; M, Moisture stress

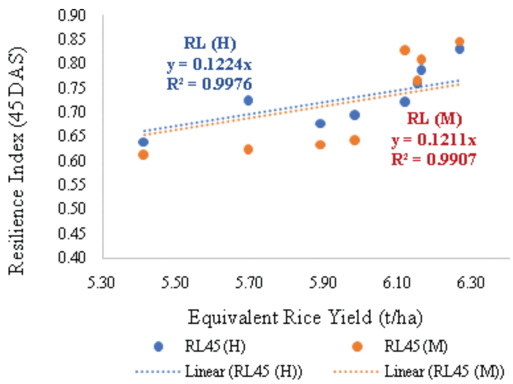

Fig. 5: Relationship between Resilience Index and Equivalent rice yield of soil under CA based R-W system RS, Resistance index; H, Heat stress; M, Moisture stress

was highest among the treatments. Fully conventional treatment was found to be least resilient to moisture stress with 61% recovery after 45 days (Fig. 3).When compared to traditionally tilled plots, zero-tilled treatments performed better in terms of the soil's resistance to moisture stress. Less soil disturbance from CA techniques results in less oxidation of organic matter, boosting SOC storage (Soni *et al.*, 2020). A plentiful supply of crop wastes used as surface mulch may have assisted in improving soil biological activity, controlling weeds and moderating soil temperature (Parihar *et al.*, 2020). Fresh crop leftovers typically serve as an ongoing labile SOM source in zero tilled treatments with residues, where they are captured during the development of macroaggregates. Here SOC have sufficient time to coalesce and go through biochemical changes that would have led to chemical recalcitrance under CA-based reduced tillage (Jat *et al.*, 2019). The variety of microbial communities and characteristics of the inherently occurring soil organisms are strongly connected with resistance and resilience.

To understand the relationship between resistance index, resilience index and yield, regression analysis was performed between them. Regression equation explained 98% variation under heat stress with r^2 value of 0.98 and 0.97 under moisture stress and indicated good agreement between resistance index and equivalent rice yield (Fig. 4). To validate resilience index, regression analysis between resilience index and management goal attribute i.e. equivalent rice yield was done. 99% agreement between recovery rate and yield was seen under heat stressed and moisture stressed soil (Fig. 5).

CONCLUSION

The novelty of the present work lies in the assessing resistance and resilience of soil microorganisms involving nutrients availability and maintaining soil health and find out the best management options under global climate change scenario. From the above study, we can conclude that under the Indo-Gangetic plain's conservation agriculture techniques-based rice-wheat farming system, highest resistance and resilience of FDA hydrolase activity was found under triple zero tillage treatment with triple residue retention after heat and moisture stress. Therefore, this practice may be recommended under CA based rice- wheat system in Indo-Gangetic plain.

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