



Resistance and resilience of soil fluorescein diacetate activity against abiotic stress

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Received: 04 January 2021; Accepted: 05 February 2021

ABSTRACT

Present study was under taken to evaluate the impact of long-term application of fertilizers and lime on fluorescein diacetate (FDA) activity, and their resistance and resilience against heat stress (48°C for 24 h) in acidic Alfisol. To accomplish this, soil samples (0-15 cm depth) were collected from seven treatments (control, 100% N, 100% NP, 100% NPK, 100% NPK+ handweeding, 100% NPK+FYM and 100% NPK+lime) from soybean crop under long-term fertiliser experiment (LTFE) at Birsa Agricultural University, Ranchi (2018). Resistance and resilience capacity of FDA were analyzed at 1, 15, 30 and 60 days after heat stress (DAS). FDA in soil was significantly higher in NPK+FYM (747 µg fluorescein g/soil/h) followed by 100% NPK+hand weeding, 100% NPK+lime, 100% NPK, 100% NP, 100% N and control treatment. At 1 DAS, maximum reduction (69%) of FDA was noticed under 100% NP alone plots followed by 100% N (64%) treatments over initial value. Treatment 100% NPK+FYM had highest resistance index (0.33) with lowest resistance (0.19) was noticed in 100% NP alone treatment. Recovery rate of most of the treatments was higher during 15 to 30 DAS and almost 100 % recovery at 60 DAS. In nutshell, FDA emerged as a good indicator to assess resistance, resilience and functional behavior of soil against abiotic stress.

Keywords: Alfisol, Heat stress, Lime, Long-term fertilization, Soil enzymes, Soil function

Soil enzymes are the catalytic agent and mediators of key soil functions, which include release of mineral nutrients for growth and development of plants; biological fixation of nitrogen; breakdown of organic matter; nitrification etc. (Vishwanath *et al.* 2020). These also play a pivotal role in carbon (β -glycosidase and β -galactosidase), nitrogen (urease), phosphorus (phosphatases), and sulphur (sulphatase) cycle (Karaca *et al.* 2010). In soil ecology, some enzymes are function as a biological indicator of the soil. Among them, fluorescein diacetate activity (FDA) is widely used as an indicator of soil biological activity (Kumawat *et al.* 2017). In soil system, it released from major groups of decomposers, bacteria and fungi etc. Resistance and resilience, of microbial community to environmental stresses (high temperature and drought) directly/indirectly influences soil functions and consequently the soil quality sustainability (Kumar *et al.* 2014). It is agreeable that resistant and resilience of soils are desirable in the context of sustainable soil management (Seybold *et al.* 1999, Kumar

et al. 2013.), as it maintain different soil functions as well as other ecological services.

Change in soil fertility patterns and yield trends in different soil type have been recorded in recent years in several long-term fertilizer experiments (Behera and Singh 2010, Vishwanath *et al.* 2020). But, these long-term experiments generated only limited data on microbial community structure, diversity, beside microbiota of soil have very important role in regulating biochemical processes, which are responsible for cycling of different nutrient, maintenance of soil health and resistance and resilience of the system against abiotic stress. Higher atmospheric temperature may cause simultaneous enhancement in soil temperature that may affect different soil microbial processes (e.g. nutrient cycling) (Kumar *et al.* 2014). Some author's reported dehydrogenase activity of soil was decreased by 28–37% when continuous long term inorganic fertilizer application was at super-optimal dose (200% NPK), whereas, urease activity of soil responded positively in the range of 43–44% (Rakshit *et al.* 2016). The objective of this study was to investigate the resistance and resilience of FDA against heat stress and their relationships with yield of soybean.

MATERIALS AND METHODS

Experimental site: The study was carried out (2018) in an ongoing long-term fertilizer experiment (LTFE) started in

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the year 1972–73 at Birsa Agricultural University, Ranchi, India. The research field is situated at longitude of 85° 19' E and latitude of 23° 17' N, about 625 m amsl and experiencing hot moist sub-humid climate. The experiment was laid out in the year of 1972 under soybean (*Glycine max*) – potato (*Solanum tuberosum*) – wheat (*Triticum aestivum*) system and it was continued till 1983–84, and there after cropping system was changed to soybean-toria (*Brassica campestris*)–wheat until 1984–85 and then a onwards soybean-wheat cropping system from 1986–87.

Treatment details: The ongoing treatments of the LTFE, consisted of 10 fertilizer treatments, out of which only seven contrasting treatments were selected for this study; control (no fertilizer, no manure), 100% N, 100% NP, 100% NPK, 100% NPK+hand weeding (100% NPK+HW), 100% NPK+FYM and 100% NPK+lime. The experiment was laid out with four replications in a randomized block design (RBD) having 100 m² individual plot size. Since 1972, control plots (without fertilizer). Recommended dose of mineral fertilizers (100% NPK) is 25:13:33 kg NPK/ha for soybean and 80:26:33 kg NPK/ha for wheat crops. Nitrogen, phosphorus and potassium nutrient were supplied through urea, diammonium phosphate, and muriate of potash fertilizers, respectively. In FYM treatment, FYM was applied @10 tonnes/ha during *kharif* of every year, while in treatment 100% NPK + lime, lime was applied in the *kharif* season of every year at @ 0.40 tonnes/ha. Based on last five years observation, average C (35%), N (1.4%), P (0.32%), S (0.2%), and Ca (0.5%) content in FYM were obtained. Pre-emergence (Imazethapyr) and post-emergence weedicide (2,4-D) was applied as per standard protocol in both soybean and wheat crops in all the treatments except in 100% NPK+HW and control where hand weeding was performed for controlling the weed during crop growth period.

Collection and preservation of soil samples: Composite soil samples from 0–15 cm depth (surface) (*i.e.* 15 random samples from each replicated plot further thoroughly mixed to make one) were collected from each plot at the pod formation stage of soybean (August 2018) crop in soybean–wheat system. Further, soil placed in open-air dry condition, sieved (2 mm mesh size), and stored at 4°C with proper tagging. The gravimetric moisture content of soil was immediately determined.

Analysis of soil samples: The initial characteristic of soil (0 Days), *i.e.* FDA activity was estimated using standard protocols (Green *et al.* 2006). For heat stress, samples were kept at 48 °C temperature for 24 hr and this particular temperature (48°C) was chosen because in the study area or in sub-humid areas of India facing very hot summer (May–June), which is very frequent under changing climate scenario. After stress samples were placed in BOD incubator at 28 ± 2°C for estimating the changes in above said properties at 1, 15, 30 and 60 days after heat stress.

Development of Resistance index (RS) and Resilience index (RL): The resistance and resilience index were determined (given below) as described by (Orwin and Wardle

2004): Resistance (RS) index was calculated as;

$$RS \text{ at } \bar{t}_0 = 1 - \frac{2|D_0|}{(C_0 + |D_0|)}$$

where, D_0 is the difference between the control (C_0) and the disturbed soil (P_0) at the end of disturbance (t_0).

This index is standardized by the control, as this takes into account differences in the amount of change that a disturbance could cause. The index for resilience (RL) was calculated as the following equation (Orwin and Wardle 2004);

$$RL \text{ at } \bar{t}_x = \frac{2|D_0|}{(|D_0| + |D_x|)} - 1$$

where, D_x is the difference between control (C_x) and disturbed soil (P_x) at the end of disturbance (t_0).

This index is standardized by the amount of change initially caused by the disturbance (D_0) as this determines the state from which it has to recover. The resistance and resilience indices ranged between -1 and +1.

Statistical analysis: All the data were analyzed using procedure proposed by (Gomez and Gomez 1984) for randomized block design of the experiment. Significance referred to in the results is $P < 0.05$. For calculation of analysis of variance (ANOVA) and Tukey's honest significant different test (Tukey HSD), SAS software was used.

RESULTS AND DISCUSSION

Long-term manuring and fertilization had significant effect on fluorescein diacetate activity (FDA) of soil (Table 1). FDA at the time of soil sampling (0 day) ranged from 503 in control to 747 (μg fluorescein g/soil/h) in 100% NPK+FYM applied treatment. Continuous application of recommended dose of balanced fertilizer along with farmyard manure (100% NPK+FYM) improved FDA in soil by 48% and 25% over control and only 100% N treatments, respectively. The FDA hydrolysis represents the activity of a group of enzymes (proteases, lipases, and esterases) secreted by bacteria, fungi and micro protista (Green *et al.* 2006). The higher population density of bacteria, fungi and actinomycetes under FYM treated soil resulted more amount of proteases, lipases, and esterase in soil ecosystem and untimely higher FDA of soil (Vishwanath *et al.* 2020). Weedicide application (100% NPK) for controlling weed infestation significantly altered the activity of FDA in soil and it was significantly reduced as compared to hand weeding treatment (100% NPK+HW). The pre-emergence herbicide imazethapyr belongs to class imidazolinones and its mode of action on cell metabolism, and inhibition of acetolactate synthase (ALS) (Saari and Mauvais 2018) that may lead to suppress the FDA. Application of lime was not effective as FYM for enhancing the hydrolytic capacity of soil, although the FDA activity of 100% NPK + Lime fertilized plots was statistically similar with 100% NPK and 100% NPK+HW plots but significantly lower than 100% NPK+FYM treated plots.

Table 1 Effect of heat stress on fluorescein diacetate activity (μg fluorescein $\text{g}/\text{soil}/\text{h}$) of soil in soybean-wheat system

Treatment	Fluorescein diacetate activity					Percent change			
	Initial	1 DAS	15 DAS	30 DAS	60 DAS	1 DAS	15 DAS	30 DAS	60 DAS
Control	503 ^E	220 ^{BCD}	290 ^E	382 ^C	495 ^E	56.2	42.3	24.0	1.59
100% N	572 ^D	205 ^{CD}	339 ^D	383 ^C	545 ^D	64.1	40.7	33.0	4.72
100% NP	583 ^D	180 ^D	325 ^D	422 ^C	556 ^D	69.1	44.2	27.6	4.63
100% NPK	633 ^C	302 ^{AB}	463 ^B	562 ^B	658 ^B	52.2	26.8	11.2	-3.94
100% NPK+HW	678 ^B	287 ^{ABC}	460 ^B	541 ^B	619 ^C	57.6	32.1	20.2	8.70
100% NPK+FYM	747 ^A	367 ^A	551 ^A	648 ^A	744 ^A	50.8	26.2	13.2	0.40
100% NPK+lime	663 ^{BC}	252 ^{BCD}	417 ^C	516 ^B	632 ^{BC}	61.9	37.1	22.1	4.67
Mean	626	259	406	493	604	58.8	35.6	21.6	3.0
SE(d) \pm	12	23	5	16	10	NA	NA	NA	NA

0 D, 1 DAS, 15 DAS, 30 DAS, and 60 DAS indicates fresh soil after sampling and days after heat stress, respectively. HW, hand weeding; FYM, farmyard manure. Means followed by same letter are not significantly different according to Tukey's honest significant difference test (at $P < 0.05$). NA-means not applicable.

The effect of heat stress on the FDA activity has been clearly observed on 1 day after stress (DAS) and the reduction in FDA (69%) was highest in 100% NP alone treatment (583 to 180 μg fluorescein $\text{g}/\text{soil}/\text{h}$) followed by 100% N (64%), 100% NPK+lime (62%), 100% NPK+HW (58%), control (56%), 100% NPK (52%) and 100% NPK+FYM (51%). Compared with the undisturbed soil (0 days), the mean activity of FDA decreased by 58% at 1 DAS. Moreover, according to Vishwanath *et al.* (2020), FDA hydrolysis can be used as an indicator of microbial activity in relation to the state of the soil environment. Above results illustrated a positive effect of balanced fertilizers application and a potential negative effect of imbalanced fertilizer application on the FDA activity against heat stress. Under continuous application of only 100% N and 100% NP fertilizers, there might have been a situation where certain types of microorganism began to predominate and microbial diversity of soil decline. The less species richness in soil could had a negative effect to resist any type of biotic or abiotic stress on soil ecosystem. As like 1 DAS, the negative impact of heat stress on activity of FDA was noticed at 15 DAS. Maximum relative rate of recovery of enzymes FDA during 1-15 days after stress was noticed (80.5%) in 100% NP alone plot (180–325 μg fluorescein $\text{g}/\text{soil}/\text{h}$) followed by 100% NPK+ lime (66%), 100% N (65%), 100% NPK+HW (60%), 100% NPK (53%), 100% NPK+FYM (50%) and control (32%). The highest recovery rate in unfertilized treatment reflected due highest decrement of FDA in unbalanced fertilized treatments than balanced fertilized treatments. Continuous application of lime increased the pH and bacterial

population density of soil (Vishwanath *et al.* 2020), hence stress impact was more pronounced and statistically similar with control as well as imbalanced fertilizer treatments. Moreover, proliferations potential of bacterial population is faster than fungus, actinomycetes and fauna population in soil that was, reflected in higher relative rate of recovery of FDA in 100% NPK+lime fertilized plots. Minimum FDA rate of recovery in unfertilized plot reflected the microbial populations residing inside the soil are in the stress condition. The data on 30 day after stress (30 DAS) revealed that 100% NP alone treatment had a highest value of recovery (134%), i.e. 180 to 422 μg fluorescein $\text{g}/\text{soil}/\text{h}$ followed by 100% NPK+lime (105%), 100% NPK+HW (89%), 100% N=NPK (86%), 100% NPK+FYM (77%) and control (74%). The 100 percent recovery was recorded at 60 days after heat stress. These results are in the line of Kumar *et al.* (2014), where they also reported almost 100% recovery in soil enzymes activity after heat stress in semi-arid Inceptisol.

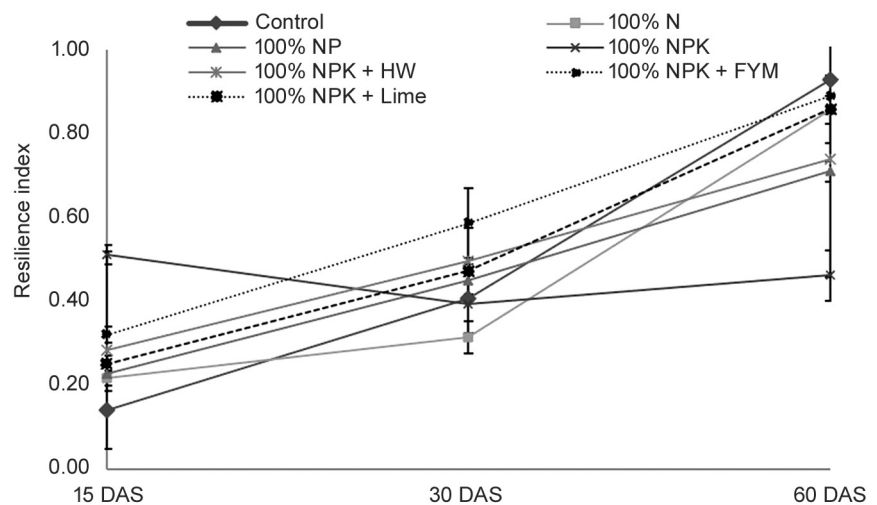


Fig 1 DAS, days after stress; Bars indicate standard errors of mean. Means followed by same letter are not significantly different according to Tukey's honest significant difference test (at $P < 0.05$).

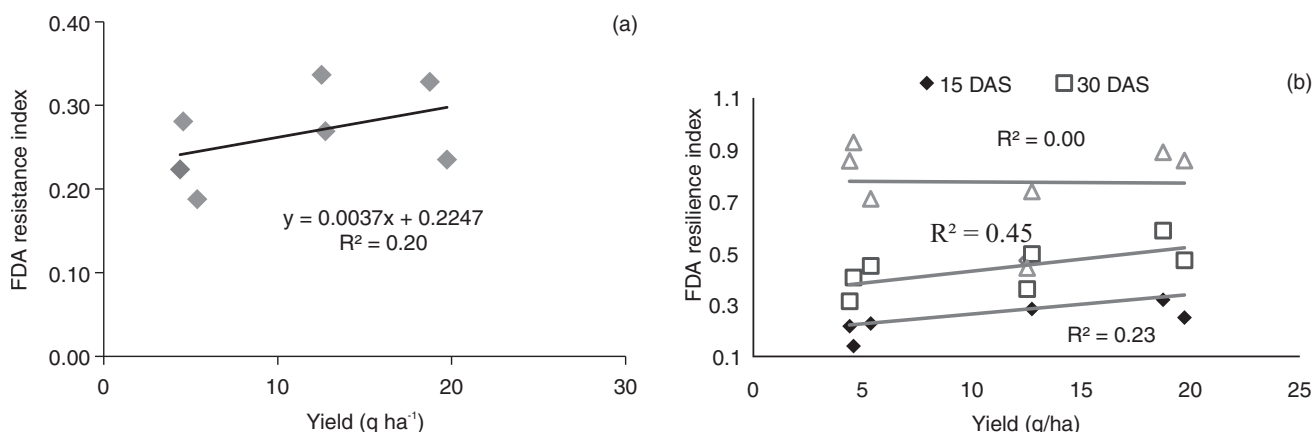


Fig 2 Relationship between resistance of soil fluorescein diacetate (FDA) with soybean yield (a), and between resilience of FDA and soybean yield at different interval (b) after heat stress of soil under long-term manuring and fertilization.

Resistance is the ability of a soil system or process to stay unchanged in the face of a particular abiotic stress/disturbance (Griffiths *et al.* 2013). The data on resistance index revealed that 100% NPK+FYM had highest resistance (RS=0.33) against heat stress which was statistically similar with the 100% NPK (RS=0.30) treatment. Among the treatments, 100% NP alone had the lowest resistance (RS=0.19) against heat stress. Adsorption of free hydrolytic FDA enzymes on to liming material provided higher thermal stability and hydrolytic stability (Rahman *et al.* 2008) and therefore providing better resistance when subjected to heat stress. From the data on resilience index (Fig 1), it was noticed that 100% NPK+FYM (RL= 0.59) and 100% NPK+lime (RL= 0.47) had higher resilience index at 30 DAS as compared to the rest of the treatments. Resilience index value also depicts recovery in all the treatments was faster during 30-60 days after stress (DAS) as compared to 15-30 days after stress (DAS). FDA activity was found to be the most resilient in 100% NPK+FYM treatment and complete recovery (RL = 0.96) was recorded at 60 DAS. It is mainly due to combined application of FYM and fertilizers could be attributed to increased microbial biomass resulting from continuous organic matter enrichment in soil (Tripathi *et al.* 2007).

The correlation between resistance as well as resilience of FDA with yield of soybean revealed very fascinating result (Fig 2). Yield of soybean and FDA activity of soil at pod formation stage of crop was significantly correlated ($r^2=0.76$) with each other. However, resistance index (high temperature, i.e. 48°C for 24 h) of soil showed less correlation ($r^2=0.20$) with the yield of crop. As time passes after heat stress the correlation between yield of crop and resilience index of soil increased (at 30 DAS, $r^2=0.45$). This clearly indicated that capacity to resist with the abiotic stress and recovery of FDA is a complex phenomenon. FDA activity of soil is a group of enzymes secreted by different types of flora and fauna in soil. It is well-known fact that regeneration potential as well as metabolic quotient of each group of microorganism is different as linked with the biochemical

oxidation-reduction process. It may be speculated that just after stress only heat tolerant microorganisms survived in the soil ecosystem, that is why low correlation coefficient was noticed. As time passes the proliferation rate of microbes increased because of ambient soil environment, that enhanced FDA and correlation coefficient with yield of soybean. However, after 60 days the resilience index of soil was not correlated with the yield of crop, this might be because of in most of the treatments 100% recovery of FDA was noticed (discussed in the previous section), which resulted RL values towards 1 in all the treatments. Result of study confer functional resilience of soils governed by the physico-chemical structure of the soil by effecting community composition and physiology of soil microbes.

From the result, it can be concluded that long-term nutrient management strongly influence the soil FDA activity, resistance and resilience capacity of soil against heat stress in an acidic environment. Imbalanced fertilization (100% N and 100% NP) and application of weedicides is frightening for functional behaviour of soil microbes as well as soil ecosystem. It was proved that fluorescein diacetate activity of soil is a good indicator to predict yield and functional behaviour of soil against heat stress.

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