



New generation post-emergence herbicides and their impact on arbuscular mycorrhizae fungal association in rice

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ABSTRACT

To prevent weed invasion in direct seeded rice cultivation, several new generation post emergence herbicides viz. bispyribac sodium, flucetosulfuron, ethoxysulfuron, fenoxaprop-p ethyl, penoxsulam, fenoxaprop-p-ethyl plus ethoxysulfuron and cyhalofop-butyl plus penoxsulam are widely used in sub-tropical rice ecosystems of Eastern India. The main objective of this study was to know whether application of above listed post emergence herbicides at recommended (n1) and double recommended dose (n2) has any negative impact on arbuscular mycorrhizal fungal (AMF) association in rice plants. Further, the effects of herbicides on soil microbial properties viz. microbial biomass carbon (MBC), fluorescein diacetate (FDA), dehydrogenase (DHA), acid phosphatase (AcP) and alkaline phosphatase (AkP) activities were analyzed using unsupervised and supervised learning methods. Results indicated that among different herbicides evaluated only application of penoxsulam significantly ($p < 0.05$) reduced the AMF root colonization (58.0%) at recommended dose (n1) compared to only AMF (70.3%) application. Whereas, application of bispyribac sodium (both n1 and n2 dose) enhanced AMF sporulation (1100 spores/100 g) and root colonization (86.68%) compared to other herbicides application. Unsupervised learning approaches through PCA found that application of bispyribac sodium enhanced both above ground plant growth responses and soil microbial properties, but penoxsulam had negative impact. But, the combined application of penoxsulam and cyhalofop-butyl did not show any negative impact on AMF association in rice plants. This study concluded that selection of right type of post-emergence herbicides are very important to minimize the harmful effect or enhance AMF association in rice plants.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) play an integral role on improvement of plant health and soil fertility (Panneerselvam et al., 2017; Chen et al., 2018; Mitra et al., 2021a). Rice a globally cultivated cereal, has been among those several recognized terrestrial plants which draws maximum benefits towards yield improvement from this symbiotic association (Panneerselvam et al., 2017, 2019). As this mutualistic relationship between AMF and plants enhance several trade-off processes existing between plant and soil, these fungal species may be regarded as ecological traders (Adeyemi et al., 2021; Mitra et al., 2021b). AMF species belonging to the phylum Glomeromycota are

obligate symbiont of plant and are helpful to several ecosystem services. Funneliformis mosseae, Rhizophagus fasciculatus, and R. intraradices are some well-known AMF species and have been encountered for improvement of crop yield in sub-tropical rice cultivation (Panneerselvam et al., 2017; Sahoo et al., 2017a, b). In rice farming practices, the application of AMF has been widely recognized for providing several ecological services such as enhancing soil health (Panneerselvam et al., 2020), reducing soil nutrient loss (Zhang et al., 2020), improving plant phosphorus uptake (Wissuwa et al., 2020), inducing resistance against phytopathogenic fungi (Huang et al., 2020), preventing drought stress in crops (Chareesri et al., 2020) and increasing crop yield (Huang et al., 2020; Sahoo et al., 2017b). Our previous studies have found the drastic

Abbreviations: AMF, arbuscular mycorrhizal fungi; SC, Suspension concentrates; EC, emulsifiable concentrate; WDG, water dispersible granule; SL, plant height; DHA, dehydrogenase activity; AcP, acid phosphatase activity; AkP, alkaline phosphatase activity; AMFc, percentage of colonization; RL, root length; BIOM, dry plant biomass; PCA, principal component analysis; AMFs, AMF sporulation density; SOC, soil organic carbon; PEG 300, polyethylene glycol; PG, propylene glycol; MEG, monoethylene glycol; DSR, direct seeding of rice.

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changes in environmental conditions particularly carbon dioxide which is a potent greenhouse gas drastically affected the diversity of AMF in soil and reduced some important commonly occurring AMF species, which could be ameliorated through reintroduction of affected species (Panneerselvam et al., 2020), thereby suggesting intermittent amendment of AMF in soil for maintaining their population and diversity.

Depending on the water regime, rice cultivation can be broadly categorized into aerobic and wetland rice systems, wherein AMF finds wide application in aerobic cropping system (Das, 2012). In aerobic cropping system growth of weeds are severe which includes *Echinochloa colona*, *E. crus-galli*, *Cyperus rotundus*, *C. irri*, *C. difformis*, *Eclipta alba*, *Celosia argentea*, *Dactyloctenium aegypticum*, *Setaria glauca*, *Scripus* spp., *Panicum repens* and *Paspalum* spp. whose concomitant growth with rice plant reduces rice yield by consuming soil nutrients (Singh, 2015). To counteract these weeds several classes of chemical herbicides are frequently used in rice fields. Some of these herbicides are potent sources of environmental stressors to soil-based microorganisms, the application of herbicide, bispyribac sodium, decreased the population of soil microbes including fungi and also affected the soil microbial properties (Kumar et al., 2020). Moreover, these prolonged changes in soil microbial health due to environmental stressors may render soil quality to decrease, thereby drastically affecting growth and development of rice plant. Modern agricultural practices have selective preferences of herbicide application, which are based on the mode of action that becomes crucial parameter for recommending large scale applicability and improvised usability of herbicides (Saiz-Rubio and Rovira-Más, 2020). Generally, the pre-emergence herbicides have broad spectrum range with some showing detrimental interactions with other living organisms. Although herbicides have been classified based on mode of action, activity site, application time, chemical groups and their persistence in soil, few studies have focused on their effect on soil microbial responses (Kumar et al., 2020). Similar to insecticides, the overuse of herbicides has also been found to drastically reduce soil microbial population and functionality (Kumar et al., 2020). Compared to pre-emergence herbicides, post-emergence herbicides have more effect on soil microbiota due to their higher rate of persistence in soil and therefore, pre-emergence herbicides such as pretilachlor and pyrazosulfuron ethyl are preferred since they do not possess long term effect on soil microorganisms (Srividhya and Ayyappan, 2020). Some examples of widely used post emergence herbicides in rice cultivation are bispyribac sodium, flucetosulfuron, ethoxysulfuron, fenoxaprop-p ethyl, penoxsulam and cyhalofop-butyl. Several combinations of herbicides such as fenoxaprop-p-ethyl plus ethoxysulfuron and cyhalofop-butyl plus penoxsulam are used since they have demonstrated proven capabilities on controlling broad spectrum of weeds (Raj and Syriac, 2015) and high rate of dissipation in soil (Sondhia, 2014). As the impact of most of these herbicides on below ground responses of mycorrhized rice plant still remains unknown, it necessitates requirement for developing a framework for analyzing the ecological responses of trade off offered by mycorrhized plants in the rhizosphere (Bernaola et al., 2018). In doing so a stoichiometric response of AMF on plant and soil responses could be established, this will be helpful for predicting the outcome of AMF interaction in relationship with other soil parameters (Liu et al., 2016). There are scientific evidences proved that application of some of the herbicides like alachlor (Pasarihu et al., 2013) and glyphosate (Helander et al., 2018) significantly reduced mycorrhizal association in crop plants and forage grasses. In rice, herbicides application is inevitable to manage weeds menaces, it is essential to document how herbicides application affects mycorrhizal association in rice plants. In view of the above, an experiment was undertaken for delineating the effects of spatial coexistence of AMF on the trade-off processes between plant and soil in responses to the profound effect of herbicides on AMF symbiosis.

2. Materials and methods

2.1. Experimental setup

The study used completely randomized block design (CRD) having two factorial levels of herbicide formulations with three replications for each treatment including positive control. Rice plants were raised under potted conditions inside net house located at ICAR-National Rice Research Institute (NRRI), Cuttack (20.4560°N and 85.9260°E), India. Each pot had volume of 5 L with 18.0 cm height, 22.5 cm top diameter and 16.5 cm base diameter and contained 3.0 kg soil, which was sterilized at 121 °C for 1hr for eliminating AMF spores. The soil used in this experiment was collected at depth of 0–20 cm from aerobic rice fields of NRRI. The initial physicochemical properties of soil collected from aerobic rice ecosystem were as follows: the texture of soil was sandy clay loam type, acidic in range (pH: 6.21), low salinity (EC: 0.45 dSm⁻¹), moderate carbon level of 0.56%, moderate total nitrogen level of 0.045%, low available phosphorous level of 12.3 kg ha⁻¹ and moderate available potash level of 153 kg ha⁻¹.

2.2. AMF inoculum development and soil treatment

Soil based AMF inoculum containing propagules of *F. mosseae*, *R. fasciculatus*, and *R. intraradices* was applied at the time of soil preparation in pots (100 g inoculum per pot). The soil-based AMF inoculum containing 90–95 spores/g soil along with mycelial fragments. In each pot ten rice seeds var. Naveen soaked in water for overnight were sown, after germination three plants were maintained in each pot till end of the experiments. As per the treatments, herbicides were applied at recommended and double the recommended dose separately through water after seventh day of sowing. Moisture content was maintained on field capacity till end of the experiment. Plant and soil samples were collected from each treatment after 60 days after sowing (DAS) for analysis of mycorrhizal root colonization, sporulation, microbial properties and plant growth parameters.

2.3. Herbicide application

The herbicides treatment on plant were arranged into eight treatments with four replications each, which included, evaluation of three types of formulations namely suspension concentrates (SC), emulsifiable concentrate (EC) and water dispersible granule (WDG). The effect of SC, EC and WDG were evaluated individually and in combination as per the compatibility of chemical pairs. To understand the effect of herbicides on AMF species, the formulations were compared with only AMF with no herbicide applications. The following are treatment details imposed in this study viz., T1: Bispyribac sodium 10% SC, T2: Flucetosulfuron 10% WDG (w/w), T3: Ethoxysulfuron 15% WDG (w/w), T4: Fenoxaprop-ethyl 6.7% EC (w/w), T5: Penoxsulam 21.7% SC, T6: fenoxaprop-ethyl 6.7% EC (w/w) + Ethoxysulfuron 15% WDG (w/w), T7: Cyhalofop-butyl 12% EC (w/v) + Penoxsulam 21.7% SC and T8: Only AMF with no herbicides. Two doses of herbicide were compared to know their influence on AMF association, which included recommended dose (n1) and double the recommended dose (n2).

2.4. Analysis of plant samples

The following parameters included plant root AMF colonization, plant height and root length and dry plant biomass were analysed after 60 days after sowing. AMF colonization was determined microscopically by examination of rice roots, which followed the standard procedure of lignin removal using KOH and staining using trypan blue stain as per McGonigle et al. (1990) and expressed as percentage of colonization (AMF_c), calculated using the formula:

Plant height (pH) and root length (RL) were measured using metric scale

$$AMF_c = (\text{number of root segments colonized} / \text{total number of root segments observed}) \times 100$$

and expressed in centimetre (cm). Total plant dry biomass (TPB) was calculated gravimetrically through constant weight measures of shoot and root maintained at 60 °C for 48 h (Tiwari et al., 2019).

2.5. Analysis of soil samples

Soil samples collected from each pot after 60 days of sowing, the soil parameters viz., AMF sporulation (AMFs), microbial biomass carbon (MBC), fluorescein diacetate assay (FDA), dehydrogenase activity (DHA), acid phosphatase (AcP) and alkaline phosphatase (AkP) were analysed (Panneerselvam et al., 2019; Nayak et al., 2016). AMFs was measured using wet sieving and decanting method following counting of AMF spores under Stereo-Zoom microscope (ZEISS Stemi 508). The soil enzymes, particularly fluorescein diacetate activity (FDA), dehydrogenase activity (DHA), acid phosphatase (AcP), and alkaline phosphatase (AkP), were determined using the ultraviolet-visible spectrophotometric procedures (Panneerselvam et al., 2019; Nayak et al., 2016). Soil FDA was estimated by following the chloroform/methanol (2:1) method (Mahapatra et al., 2017), DHA was estimated by determining the reduction of triphenyl tetrazolium chloride (Chatterjee et al., 2018), and AcP & AkP, were estimated by monitoring their activities in universal buffers maintained at pH 6.5 and 11.5, respectively (S. Sahoo et al., 2017b).

2.6. Statistical analysis

Using boxplots, the variations between treatments and their corresponding replicated values, were studied. Three replications of each sample were used for estimation of individual parameters. The dotted lines in the boxplots represented the mean variance of parameter measured. ANOVA was conducted to understand the variations between levels of herbicide doses on the parameters studied. The significance levels were compared using the Duncan's multiple range test having critical difference at CD = 0.05. The statistical learning methods used for analyzing the treatment responses included both the supervised and unsupervised learning techniques, which were used for predicting the effect of herbicide treatments on both the above and below ground parameters. For supervised learning techniques, multiple linear regression models were constructed using the 'lm' function for understanding the relationship between variables. With an assumption, $f(x)$ to be linear, the coefficients (β) for the following linear equation were derived: $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n + \epsilon$, in which the shoot dry weight (SDW) and root dry weight (RDW) were used separately as response variables in two models, indicated by 'y', the model intercept was represented by ' β_0 ', and the regression coefficients represented by ' β_{1-n} ' were calculated from respective ' x_{1-n} ' predictor variables which included root mycorrhizal colonization, soil microbiological and enzymatic parameters, and ' ϵ ' represented error of the model. Step wise regression methodology was primarily adapted to simplify the model and was computed using both forward and backward directions present in the stepAIC function of the R package MASS. Different training and testing datasets were constructed using random sampling methods and respective models were constructed. Following which the predictive models were evaluated and used for estimation of the responses of below ground parameters on total plant dry biomass and the RMS (root mean square) error calculated to find differences between predicted and actual biomass using the formulae mentioned below:

$$RMSError = \sqrt{\frac{1}{n} \sum_{i=1}^n (\text{predicted} - \text{actual})^2}$$

For supervised learning a single predictive linear mode, which had previously been verified through step regression models with 1000 bootstrap value was used. Unsupervised learning techniques involved principal component analysis (PCA) which was conducted using the prcomp function underlying the principles of single value decomposition and projection of eigenvectors over principal components through matrix multiplication. The dimensionality reduction of the transformed matrix was represented in form of biplots using the packages FactoMineR and factoextra, which illustrated the clustering of parameters represented using vector lines and clustering of treatment types represented using points. The statistical analysis including supervised and unsupervised learning methods were carried using the R software version 3.3.0 (Team, 2016).

3. Results

3.1. Effect of herbicides on root colonization and sporulation of AMF

Herbicides application on rice demonstrated considerable variations in root colonization and sporulation. Illustrated in Fig. 1 the Arc sin transformed values of AMF colonization showed a significant ($p < 0.05$) reduction in colonization in plants treated with penoxsulam (57.0% at n1 dose and 42.3% at n2 dose) as compared to the control (80.0%). Similar reduction was also observed in flucetosulfuron, which demonstrated significant ($p < 0.05$) reduction of AMF colonization (65.7%). Comparatively, application of bispyribac sodium demonstrated increment in colonization as observed in n1 (82.0%) and n2 (86.7%) doses (Supplementary Fig. 1). The impact of herbicides on AMF sporulation as illustrated in Fig. 2, revealed among the seven herbicide treatments, penoxsulam (T5) and flucetosulfuron (T2) significantly ($p < 0.05$) reduced sporulation. Compared to the control (860 spores/100 g soil), T5 recorded 762 spores/100 g soil under n1 dose and 720 spores/100 g soil under n2 dose, followed by T2 which recorded 822 spores/100 g soil under n1 and 783 spores/100 g soil under n2, respectively. Application of bispyribac sodium (T1) significantly ($p < 0.05$) improved sporulation with 1019 spores/100 g soil and 1077 spores/100 g soil at n1 and n2 dose, respectively (Supplementary Fig. 2).

3.2. Changes in plant agronomic parameters associated with herbicide application

The following agronomic parameters were evaluated viz. plant height, root length and plant biomass (Table 1) to understand the impact of herbicides on trade-off processes associated with AMF with plant. There was no significant variation in plant height among treatments between n1 and n2 dose, but significant variation was observed in root length and plant biomass, which were more pronounced under n2 dose. At recommended dose, there was no significant variation in root length among treatments except penoxsulam treated plants, but the significant reduction of root length was noticed in T2, T5 and T7 under n2 dose. The n1 dose of herbicide did not show any variation in plant biomass as compared to only AMF application (T8), but, under double dose of herbicide application, significant ($p < 0.05$) reduction in plant biomass were observed particularly in penoxsulam (T5) and flucetosulfuron treatment (T2) having 5.35 g/plant and 5.70 g/plant dry plant biomass, respectively.

3.3. Effect of herbicides on soil microbial and enzymatic parameters

As mentioned in Table 2, among different herbicides application,

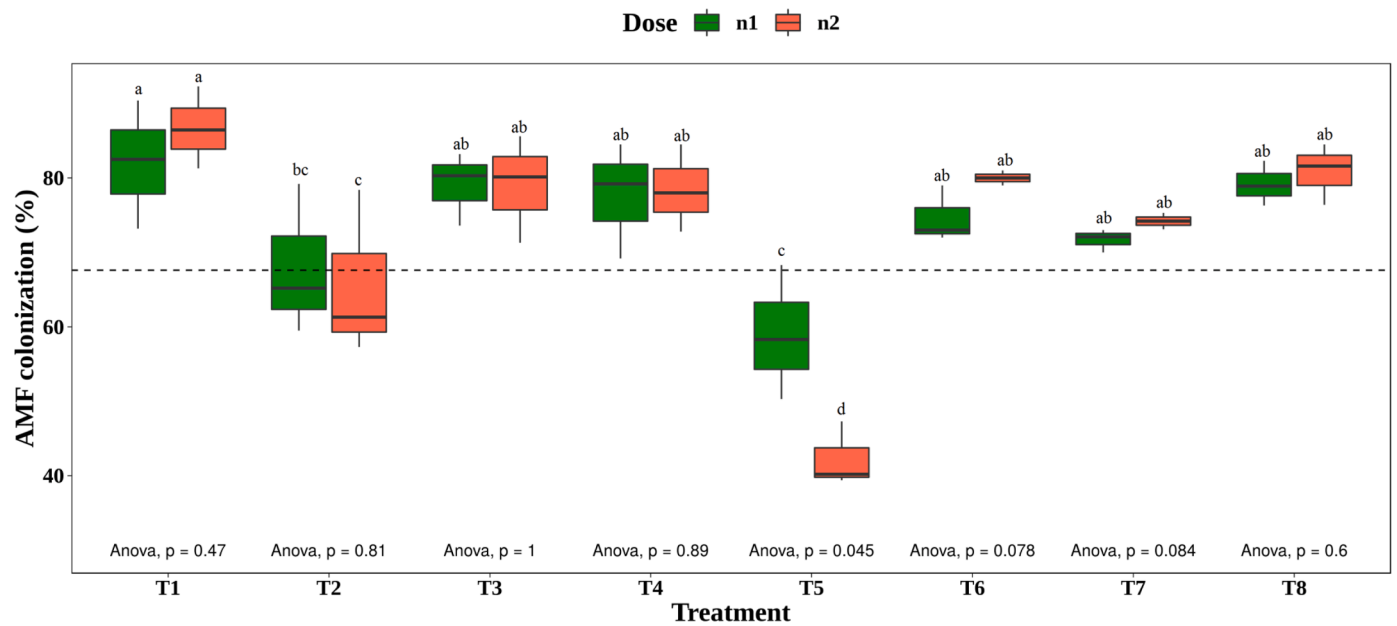


Fig. 1. Effect of different herbicides application on AMF root colonization in rice plants.

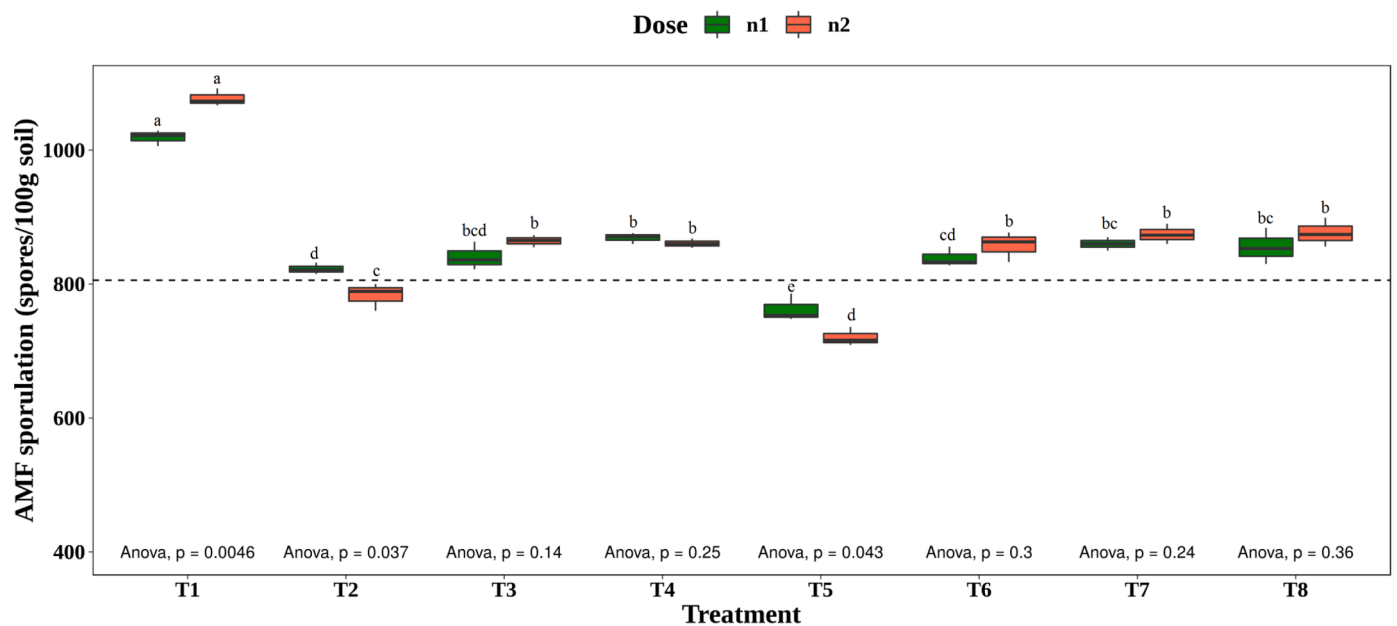


Fig. 2. Effect of different herbicides application on AMF sporulations in rice rhizosphere.

bispyribac sodium treatments either n1 or n2 dose resulted in consistent improvement in MBC, DHA, FDA, AcP and AkP compared to other herbicides application, whereas the penoxsulam (T5) and flucetosulfuron (T2) application significantly reduced these parameters as compared to only AMF application (T8). In penoxsulam plus cyhalofop-butyl (T7) application recorded significantly higher MBC ($540.34\text{--}549.23 \mu\text{g}\cdot\text{g}^{-1} \text{soil}\cdot\text{h}^{-1}$), DHA ($21.0\text{--}21.47 \mu\text{g TPF}\cdot\text{g}^{-1} \text{soil}\cdot\text{h}^{-1}$), FDA ($34.03\text{--}35.07 \mu\text{g}\cdot\text{g}^{-1} \text{soil}\cdot\text{h}^{-1}$), AcP ($37.0\text{--}37.47 \mu\text{g p-nitrophenol released}\cdot\text{g}^{-1} \text{soil}\cdot\text{h}^{-1}$) and AkP ($33.83\text{--}34.30 \mu\text{g p-nitrophenol released}\cdot\text{g}^{-1} \text{soil}\cdot\text{h}^{-1}$) as compared to individual (T5) application of penoxsulam, but this trend was not observed in ethoxysulfuron plus fenoxaprop-p ethyl application. In general, the higher dose of herbicide application did not show much variation in microbial properties between n1 and n2 dose except penoxsulam (T5) and flucetosulfuron treatment.

3.4. Unsupervised learning using PCA for understanding the patterns associated with changes in plant and soil parameters with respect to doubling of recommended herbicide dose

PCA conducted separately on n1 and n2 herbicide dose application datasets revealed the total variance was higher in n2 (81.5%) as compared to n1 (71.8%), depicting the higher dose of certain herbicide applications had significant impacts on the response parameters. To identify those parameter and treatment variables, biplot was conducted. The variation of parameters across different treatments under n1 dose has been presented in Fig. 3 from which it could be deciphered the responses of all parameters were higher in T1 and T8, whereas the responses were lower in T5 followed by T2. The variations in parameters due to increase in recommended dose of application has been represented in Fig. 4 which stated similar variations as compared to n1 dose

Table. 1

Effect of different dose of herbicide applications on responses of agronomic parameters in relationship with mycorrhization of rice plants with AMF species evaluated after 60 DAS.

Treatment	Plant Height (cm)		Root Length (cm)		Plant Biomass (gm dry weight/plant)	
	n1	n2	n1	n2	n1	n2
T1	69.03	69.86	16.13 ^a	16.85 ^a	6.20	6.17 ^{abc}
T2	65.59	67.23	16.10 ^a	14.67 ^{de}	5.85	5.70 ^{cd}
T3	67.99	67.55	15.93 ^a	15.74 ^{bc}	6.03	6.16 ^{abc}
T4	66.56	68.03	15.94 ^a	16.14 ^{abc}	5.69	6.06 ^{abc}
T5	61.53	67.83	14.90 ^b	14.36 ^e	5.69	5.35 ^d
T6	66.03	68.13	15.70 ^a	15.77 ^{bc}	6.09	6.19 ^{abc}
T7	67.53	68.70	15.70 ^a	15.40 ^{cd}	6.16	6.48 ^{ab}
T8	70.45	70.51	16.36 ^a	16.47 ^{ab}	6.46	6.60 ^a
CD(0.05)	ns	ns	0.72	0.87	ns	0.62

Similar alphabets corresponding to different numbers represent treatments which do not have significantly variance analyzed at 5% level of significance. ns: non-significant.

demonstrating higher in T1 and T8 whereas lower variations in T5 and T2. Among all the response parameters studied, the following three clusters based on parameters relationships were identified. In the first PCA biplot representing n1 application dose, the respective parameters could be clustered, starting with the first cluster represented by FDA, AcP, MBC, which were higher than 0.85 correlation coefficient ($p < 0.001$), the second cluster represented by AkP, pH and AMFc which were lower than 0.85, but higher than 0.80 at $p < 0.001$ and the third cluster represented by RL, AMFs and TPB which were less than 0.80 but higher than 0.70. Similarly, for the PCA biplot represented for n2 response dataset, three types of clusters could be identified. The first cluster consisted of FDA, AMFc and RL, which were found to have maximum correlation greater than 0.90, followed by DHA, AMFs, MBC, AcP and AkP which had correlation greater than 0.80 and the final cluster consisting of TPB and pH which had correlation greater than 0.70.

3.5. Supervised learning using predictive structural linear models using below ground responses

Based on supervised learning strategies, two models one based on prediction of standard deviation of weighted sum of variables (SDW) and another on reporting data warehouse (RDW) was carried separately and their responses were combined to predict the total dry biomass. The following models were developed and evaluated for predicting SDW and RDW.

$$SDW = 1.746 - ; 0.007(AMFc) - 0.004(MBC) + 0.11(FDA) + 0.01(DHA) + 0.048(AcP) + 0.003(AkP)$$

Table. 2

Comparisons of changes associated with below microbial and enzymatic soil parameters observed in rhizosphere soil samples of plants treated with different herbicides under recommended and double dose of herbicides evaluated after 60 DAS.

Treatment	MBC ($\mu\text{g}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)		DHA ($\mu\text{g TPF}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)		FDA ($\mu\text{g}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)		AcP ($\mu\text{g p-nitrophenol released}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)		AkP ($\mu\text{g p-nitrophenol released}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$)	
	n1	n2	n1	n2	n1	n2	n1	n2	n1	n2
T1	549.83 ^{ab}	549.10 ^{ab}	22.51	23.17 ^a	34.92 ^a	36.46 ^a	39.58 ^a	41.81 ^a	34.90 ^a	36.79 ^a
T2	531.00 ^{cd}	513.86 ^c	20.93	20.00 ^e	33.01 ^b	31.33 ^c	35.27 ^{bc}	31.23 ^e	32.63 ^b	25.53 ^e
T3	539.33 ^{bc}	533.67 ^{bc}	15.33	21.03 ^{cd}	34.09 ^a	34.14 ^b	36.27 ^b	38.92 ^b	33.67 ^{ab}	33.31 ^b
T4	545.00 ^{abc}	544.33 ^{abc}	21.66	21.70 ^{bc}	34.07 ^a	34.02 ^b	36.50 ^b	36.94 ^c	32.13 ^b	31.42 ^{bc}
T5	514.88 ^d	484.87 ^d	20.03	18.82 ^f	29.77 ^c	28.92 ^d	33.80 ^c	32.50 ^{de}	28.13 ^c	26.10 ^{de}
T6	533.71 ^{bc}	538.67 ^{ab}	21.81	21.73 ^b	34.00 ^{ab}	34.17 ^b	33.37 ^c	33.37 ^d	28.87 ^c	29.20 ^{cd}
T7	540.34 ^{bc}	549.23 ^{ab}	21.00	21.47 ^{bcd}	34.03 ^{ab}	35.07 ^{ab}	37.00 ^b	37.47 ^{bc}	33.83 ^{ab}	34.30 ^{ab}
T8	558.45 ^a	558.63 ^a	20.75	23.06 ^a	34.93 ^a	35.20 ^{ab}	40.03 ^a	40.30 ^a	34.80 ^a	35.83 ^a
CD(0.05)	16.94	22.98	ns	0.70	1.06	1.49	2.01	1.78	1.98	3.17

MBC: microbial biomass carbon, FDA: fluorescein diacetate activity, DHA: dehydrogenase activity, AcP: acid phosphatase and AkP: alkaline phosphatase ns: non-significant. Similar alphabets corresponding to different numbers represent treatments which do not have significantly variance analyzed at 5% level of significance.

$$RDW = 0.87 + 0.002(AMFc) - 0.001(MBC) + 0.025(FDA) + 0.002(DHA) - 0.005(AcP) + 0.012(AkP)$$

With total 41 degrees of freedom (df) SDW had $R^2 = 0.563$ ($p < 0.001$) whereas RDW had $R^2 = 0.787$ ($p < 0.001$). From the results obtained it was found SDW had higher correlation with FDA having $\text{Pr}(> |t|)$ value of 0.023 and AcP having $\text{Pr}(> |t|)$ value of 0.037 which were both significant at $p < 0.01$. Similarly, RDW had higher correlations with AMFc having $\text{Pr}(> |t|)$ value of 0.037, MBC having $\text{Pr}(> |t|)$ value of 0.025, FDA having $\text{Pr}(> |t|)$ value of 0.012 and AkP having $\text{Pr}(> |t|)$ value of 0.003 significant at $p < 0.01$ level. The predicted biomass values are represented in Fig. 5, which showed significant reductions in predicted biomass associated with T2, T5 and T6. From these predictions, it was inferred that although some combinations of herbicides like penoxsulam with cyhalofop-butyl although could reduce the negative effect associated with individual application of penoxsulam, but certain combinations like fenoxaprop-p-ethyl with ethoxysulfuron at n2 dose, were found to have negative impact on below ground responses as compared to individual applications of fenoxaprop-p-ethyl and ethoxysulfuron. Applying the formulae as given in the statistical methodology, the RMS error between predicted and observed plant dry biomass was computed to be 0.478 guaranteeing correctness of the model applied.

4. Discussion

Direct seeding of rice (DSR) under aerobic conditions in soil provides an opportunity for reducing water application, methane gas emission and labor requirement (Pathak et al., 2013). However, under sub-tropical humid climates, severe weeds invasion in aerobic soil is a common phenomenon observed in rice ecosystem. To prevent the competition of rice plant against weeds, a wide range of herbicides have been developed. Continuous as well as indiscriminate applications of herbicides have caused genetic changes, resulting in evolution of herbicide resistant weed species and thus reduce the efficacy of herbicides for controlling target weeds. Therefore, new generation herbicides with different formulations have been developed for weed management practices. To most extent the effect of next generation herbicides on AMF, have had remained obscure until now. Earlier, glyphosate based herbicides have been found to reduce mycorrhization upto 40% in soils amended with *F. mosseae* due to alteration in AMF phenology, and have also stated the impact of same type of herbicide to vary across different species of AMF (Zaller et al., 2014). The present study has found similar results of reduced mycorrhization in plants treated with penoxsulam formulation. AMF identified as important players for translocation of photosynthates of plants to soil, this mutualism improves soil organic carbon (SOC) content (Panneerselvam et al., 2020). The increase in soil SOC content has been identified a crucial step in the methods of regenerative agriculture that has potential of improving soil structure

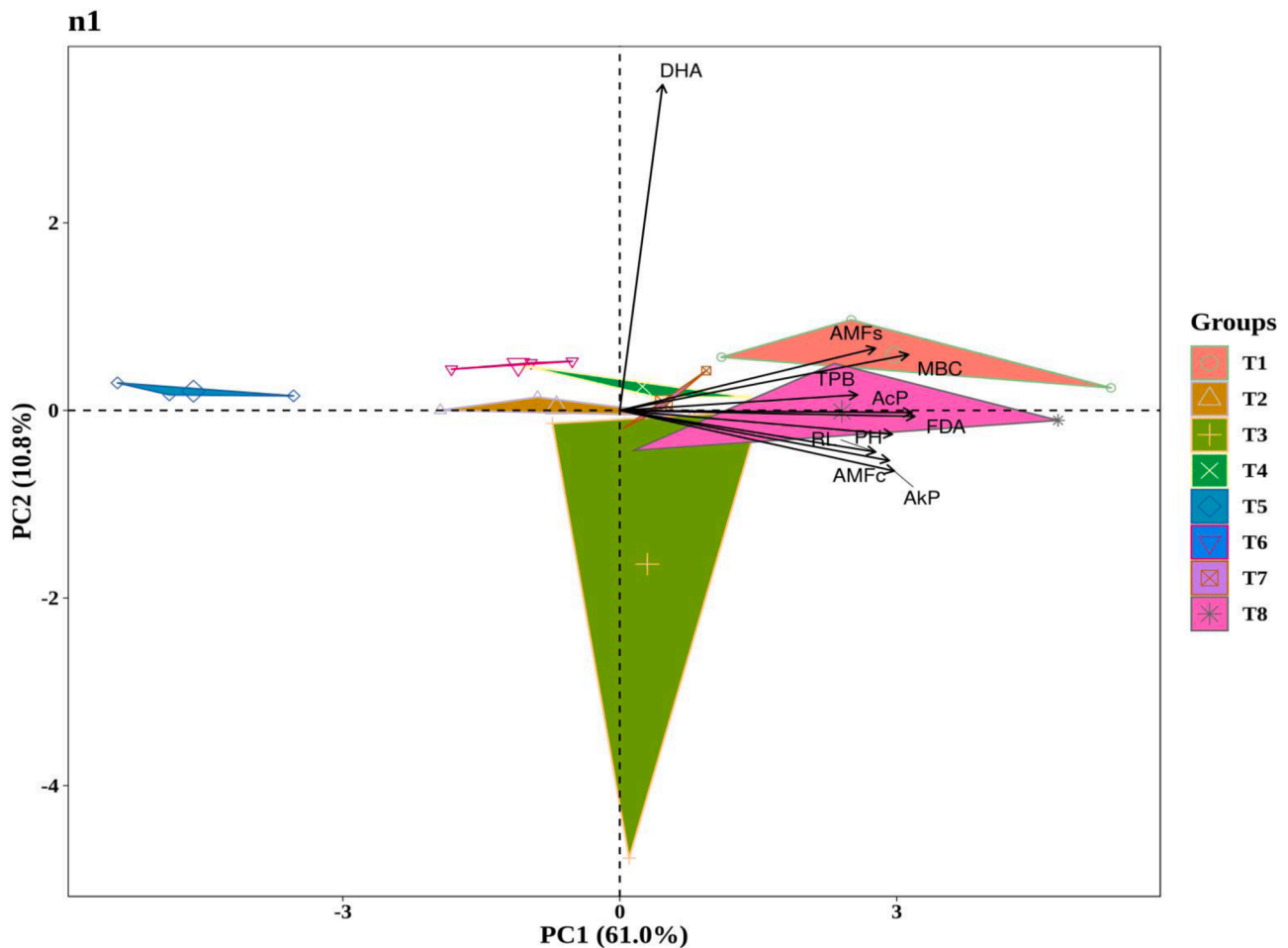


Fig. 3. Representation of PCA results using biplots for analyzing responses of plant growth parameters and microbial properties estimated under n1 dose level of herbicides.

and health along with amelioration of soil edaphic stress cause due to flooding, drought and erosion of soil (Rhodes, 2017). However, the detrimental consequences of herbicides on mycorrhization have alarmed the scientific community on assessment and necessary measures to be taken towards sustaining AMF species in soil.

In this study a combination of supervised and unsupervised computational algorithms were used to understand the impact of herbicides and their doses of application on AMF functionality and associated trade-offs. As described earlier by Schröder and Kern (2018), deep learning could be categorized into unsupervised and supervised learning methodologies, an example of which is PCA which is an unsupervised learning technique i.e. capable of clustering similar kind of relationships between variables. From the PCA analysis it was inferred that there was significant effect on soil FDA activity. Previous research has revealed AMF colonization in rice plants had demonstrated significant ($p < 0.05$) increase in MBC and soil enzymes including FDA, DHA, AcP and AkP (Panneerselvam et al., 2019). However, in the current study the application of certain herbicides including penoxsulam, flucetosulfuron, fenoxaprop-p-ethyl and ethoxysulfuron are capable of interacting with AMF metabolism thereby reducing their effectiveness as demonstrated from the significant reduction in parameters values of soil microbial and enzymatic properties. Studies have found presence of acetolactate synthase (ALS) in microbes including plants and fungi, and thus are prone towards, ALS inhibiting post emergence herbicides (Karpouzias et al., 2014). Penoxsulam, flucetosulfuron and ethoxysulfuron belong to the class of ALS inhibitors, which might be responsible for decrease in AMF and soil associated microbial activity. Similarly, fenoxaprop-p-ethyl

have been found to suppresses the biosynthesis of fatty acids by inhibiting acetyl CoA carboxylase enzyme (Kim et al., 2005) and also have been reported to negatively affect AMF and other soil mycoflora (Gupta et al., 2011). Similar to previous research outcomes, the current work also deciphered that the application of these three herbicides to be harmful to AMF and soil microflora. Moreover, the supervised learning techniques carried to classify the predicting target variable i.e. total plant biomass and predictor response variables viz. plant and soil parameters, demonstrated combination of penoxsulam with cyhalofop-butyl was better than individual application of penoxsulam. Whereas combined application of fenoxaprop-p-ethyl with ethoxysulfuron had negative impact on below ground responses.

Through this experimental work, the difference in colonization and sporulation potential due to the effect of two kinds of herbicides formulations viz. liquid sprayable (SC and EC), and dry sprayable (WDG) were quantified. Combination of EC with SC and WDG were also tested and compared against AMF treated plants. Compared to the different kinds of formulations mentioned, the tested herbicide with EC formulation have been regarded to be toxic than SC and WDG. A brief description of different kinds of herbicide formulation has been provided by Hazra and Purkait (2019). Several chemical ingredients are generally used for preparation of herbicides viz. surfactants, buffering agents, antifoam agents, preservative chemicals, acid scavengers, emulsifiers, dyes, antifreeze agents, dispersants densifiers, suspending agents, and crystal promoters. Among which the surfactants are widely universally used for preparation of herbicides which aids in uniform dispersal and penetration of herbicides by dissolving the waxy layer

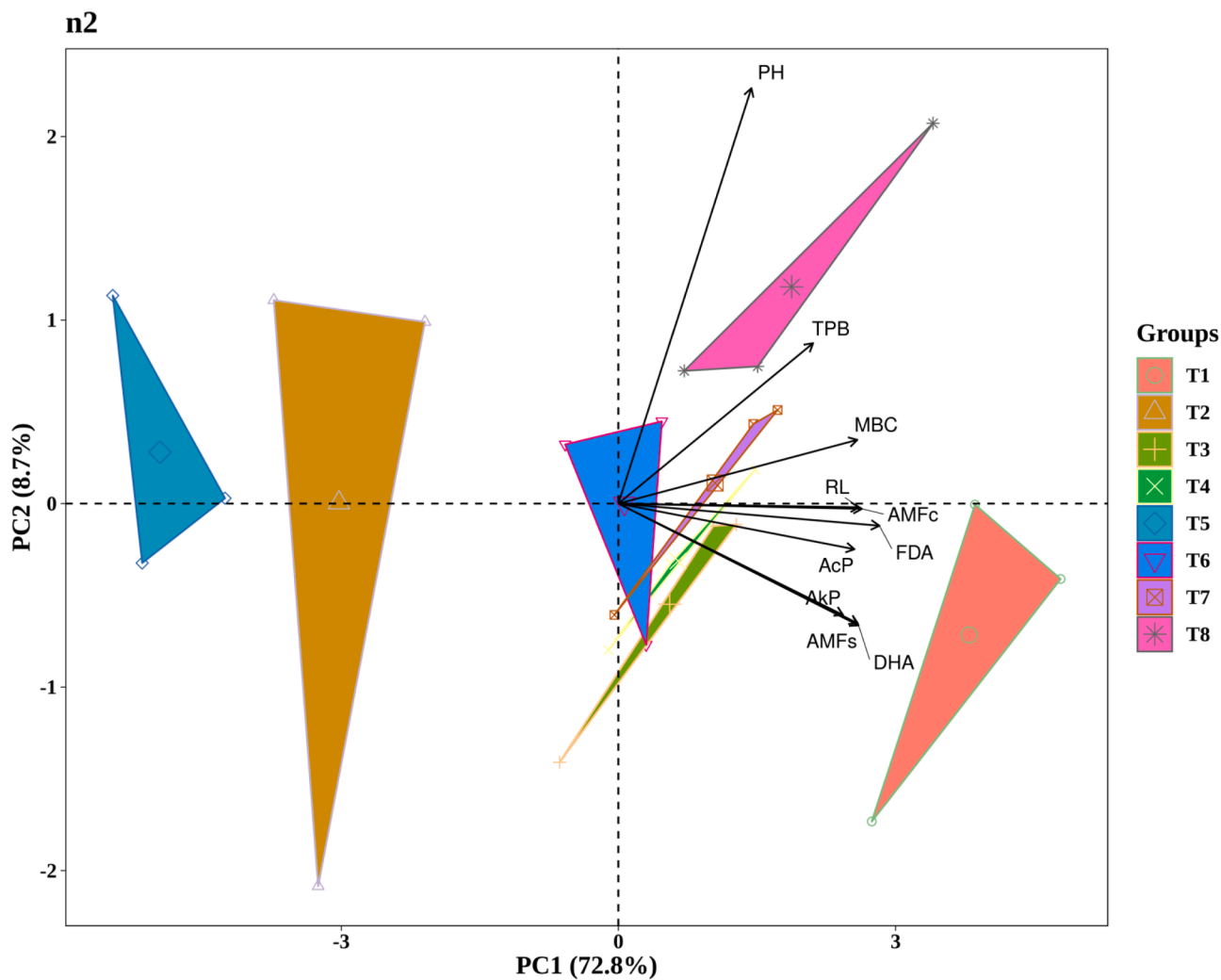


Fig. 4. Representation of PCA results using biplots for analyzing responses of plant growth parameters and microbial properties estimated under n2 dose level.

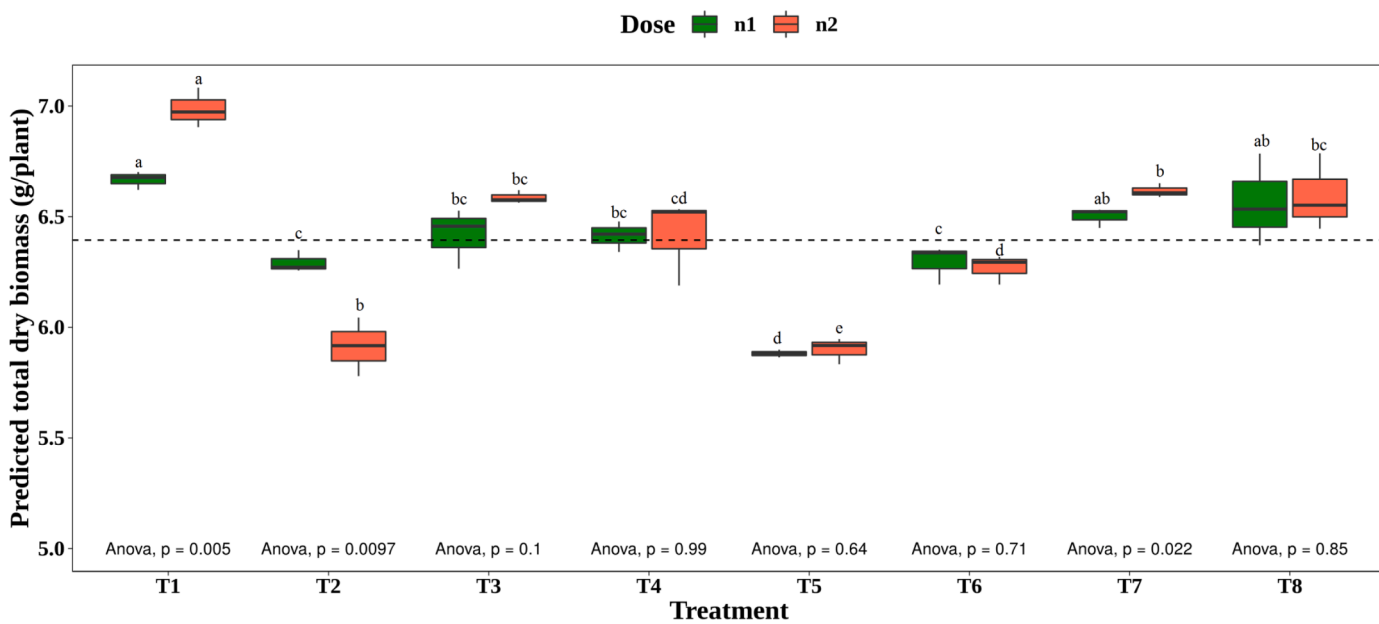


Fig. 5. Comparison of plant biomass obtained from predictive models using below ground response parameters.

present in leaf cuticle (Tominack and Tominack, 2000). Studies have found several surfactants associated with formulation of herbicides have cytotoxic effects (Song et al., 2012), known as surfactant toxicity which causes damages to cell membrane, thereby disturbing cellular metabolism, with severity being seen in case of polyoxyethylene tallow amine (TN-20), polyoxyethylene lauryl amine ether (LN-10), and poly (oxyethylene, oxypropylene) glycol block copolymer (PE-61) based herbicide formulations, as compared to polyethylene glycol (PEG 300), propylene glycol (PG), and monoethylene glycol (MEG), which have been found to act as nontoxic surfactant agents. Additionally, soil-based microorganisms have been found to modify the chemical structure of surfactants and reduce their toxic effect on environment (Ying, 2006). For which an increase in soil microbial activities under higher doses of herbicides was observed.

Based on the present findings, it was found the herbicide treatments particularly SC formulated bispyribac sodium, stimulated AMF colonization, sporulation and other soil microbial properties. This observation was in contrast to previous findings which showed double dose of bispyribac sodium to be harmful to soil microbial community (Kumar et al., 2020), but however had not taken into account the functional dynamics of AMF in soil. Although flucetosulfuron 10% WDG have been found to effectively control weed without showing any symptoms of phytotoxicity in preceding crops under direct seed rice system (Sridhara et al., 2019), our findings showed this herbicide category reduced the AMF colonization potential in rice roots. Related experimental findings have found decrease in anastomosis formation in *F. mosseae* due to the effect of low levels of herbicides, which results in retardation of AMF growth and disruption of mycelial structure, thereby threatening AMF symbiosis in plants and their associated trade-off processes (de Novais et al., 2019).

Our previous works have identified AMF to play an important role on improvement of structure and function of microbial community in rice ecosystem (Panneerselvam et al., 2020). Therefore, in order to effectively analyze the impact of herbicides on the soil microbial community, the microbial functionality in soil was analysed by estimating the responses of FDA and DHA. Combinations of EC and WDG formulations having fenoxaprop-p-ethyl 6.7% (w/w) and ethoxysulfuron 15% (w/w) showed reduction in soil microbial function and community structure. Earlier research on wheat, have also found fenoxaprop-p-ethyl to have reduced the microbial population in soil and also have affected root colonization and sporulation potential of AMF (Gupta et al., 2011). In the present study, it was noticed that penoxsulam also registered deleterious effect on soil microbial properties as well as AMF association, but, these negative effects were significantly reduced when it combined with cyhalofop-butyl application. Previously the combinations of penoxsulam and cyhalofop-butyl have been found to suppress fungal growth and with increase in the concentration the suppression was more severe (Raj et al., 2018). The combination of penoxsulam and cyhalofop-butyl have been found effective on reduction of weed menace in DSR (Patil et al., 2016), we found the same combination did not show any significant reduction in AMF association and other microbial properties as compared to only AMF application, which is one of the positive sign for preservation of soil microbial health under weeds management approach. From the above observation, it was inferred that the harsh effect of some individual herbicide application towards soil microbial properties including AMF association can be minimized when it combined with other compatible herbicides.

Moreover, recent findings suggest the composition of fungal community has been found to be determined by the plant genotype and the modulation of fungal population is affected by concentration of phosphate in soil (Zuccaro, 2020). Therefore, in addition to the herbicide effect on AMF, the trade-off between AMF and plant is also controlled by the plant genotype and phosphate concentration in soil. Perhaps the decrease in weed population due to application of herbicides might have resulted for the increase of trade-off processes, but the toxic effects of herbicide formulation should never be ignored during understanding of AMF dynamics in ecosystem. We speculate the role of surfactant and

other substances present in herbicides may be responsible for toxic effect causing disturbances in AMF dynamics between plant and soil. Hence, the use of right type of herbicides or its combination is very essential to improve microbial properties particularly AMF association in rice soils.

Conclusion and future prospects

After evaluation of different groups of herbicides and its combinations, using supervised and unsupervised learning methods, it was found that the bispyribac sodium based soluble concentrate formulation improved the AMF colonization, sporulation and other microbial properties. Similarly, the following herbicides i.e. ethoxysulfuron and fenoxaprop-p ethyl application did not show negative effect for AMF association in rice. Some individual herbicides application i.e. penoxsulam and flucetosulfuron reduced the AMF association and other rhizosphere microbial properties in rice. But, the negative effect of mycorrhizae trade-off in rice due to individual application of penoxsulam could be prevented by combination with cyhalofop-butyl. From this present study, it was inferred that selection of right type of post emergence of herbicides are very essential to reap the complete beneficial effect from AMF application in rice cultivation.

Author declaration

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Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

Credit author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the "Current Research in Microbial Sciences".

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Supplementary materials

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