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Effects of Bacteria on the Yield and Quality of Spring Barley

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Abstract **– Bacterial formulations have been widely used to improve recycle of nutrients and waste in several sectors including agriculture. This study is conducted to compare the effects of two commercial waste degraders (ACF-32, ACF-SA) on barley productivity in comparison with standard farm practice. This trial was carried out in a randomised design with three replicates. Treatments were control (standard farm practice), ACF-32 treated plants and ACF-SA treated plants. The results showed a significant difference between the treatment with ACF-SA or ACF-32 and standard farm practice where the treatment of barley with ACF-SA performed better than the control and ACF-32. Particularly, protein, nitrogen, phosphorus, potassium and zinc content were higher reflecting an increased yield of 1.94 t/ha for barley treated with ACF-SA than those under standard farm practice.**

Keywords **– Crop Enhancement, Soil Bacteria, Bio-Fertiliser, Barely and yield.**

I. INTRODUCTION

Barley (*Hordeum Vulgare L.*) is one of the most important food crops produced in the world. It assumes the fourth position in total cereal production in the world after wheat, rice and maize [1]. Despite, the importance of barely and its many useful characteristics, several factors are affecting its production. The most important factors that reduce the yield of barley are poor soil fertility, water logging, drought, frost, soil acidity, diseases and insects, and weed competition [2]. With focus on soil fertility microbes play an important role in promoting plant health and growth as bio-fertilisation [3]. The ability of micro-organisms to promote growth and increase yield has significant economic and environmental benefits including increased income from reduced fertiliser cost [4]. Numbers of different bacteria promote plant growth, including aerobic, facultative anaerobic, chemotropic and photosynthetic species [5-7].

There are several products in the global market under the classification of "waste degrader" in response to global regulatory legislations such as; Urban Waste Water Treatment Directive (1991) [8], Water Framework Directive (2000) [9], Environmental Protection Agency (EPA) and Clean Water Act (1977) [10]. Recently, Direct Toxicity Assessment (DTA) and Whole Effluent Toxicity (WET) [11-12] tests have enforced to ensure there are no hazards entering plants. Further, biological waste degraders have been recognized and approved as environmentally safe products. The biological waste degraders include similar species of bacteria as in bio-fertilisers however their use in agriculture has not been studied widely for their effect in crop productivity.

This study investigated the efficacy of two commercial bacterial products on spring barley. Both products contained class 1 bacteria which are typically used to break down compounds to present nutrients in a more available form. Both products namely Aqua Clean™ - ACF - 32 (ACF-32) and Aqua Clean™ - ACF-SA (ACF-SA) have multiple applications outside of agriculture and are in this study tested as organic fertilisers/ bio-fertiliser. The first product, ACF-32, is a multi-purpose formula, while the second one, ACF-SA, is an organic solid waste degrader which speeds up biological oxidation of organic waste solids.

II. MATERIAL AND METHODS

The experiment was designed, performed and evaluated by Crop Intellect Ltd (Lincoln, LN2 2LG, UK) to GEP standards. Two approved biological waste degraders provided by Nutrel Products Ltd (Lincoln, LN1 2LD, UK) namely ACF-SA and ACF-32 were evaluated for application in agriculture using spring barley. ACF-32 is a very stable bacterial cultures designed for use in waste water systems as microbial treatment and contains vegetative bacteria from different species representing aerobic, anaerobic, facultative, chemo-synthetic and photo-synthetic species, and it uses biological activity to dissolve solids and it is not hazardous, toxic or harmful to humans, animals or fish and plants. ACF-SA is a natural product, non-pathogenic and with no genetically modified organisms and contains aerobic, facultative anaerobic, chemotropic and photosynthetic species, blended with organic certified humic acid.

The experiment took place in a commercial farm North of Lincoln with each treatment replicated three times. Each plot was 100m2 to ensure that field variation within plots is averaged out and a randomised block design was utilised.

Soil properties, Experimental Field Design

The experiment was performed within a field of 16.35ha at Lodge Farm Ltd. At the start of the experiment, the main characteristics of the soil used for growing barley analysed by third party certified laboratory and are presented in Table 1. The soil was loamy with the following description from the national soil survey "Shallow well drained brashy calcareous fine loamy soils over limestone" and "Free draining permeable soils on 'brashy' or dolomitic limestone substrates with high permeability and moderate storage".

The field received typical herbicides mixed effectively as a good practice for control of a wide variety of weeds and also to avoid tolerance. Nitrogen and TSP were used as basic fertiliser and for foliar elements received Magnesium, Manganese and a multi-nutrient mix. A growth regulator a-

-nd fungicides were used as standard practice for the area.

Table 1. Soil analysis of the experimental field area.

Soil test parameter	pH
pH	7.8
Copper (EDTA extractable) mg/L	2.6
Boron (hot water-soluble) mg/L	1.2
Sodium ammonium nitrate extractable) mg/L	7.0
Zinc (EDTA extractable) mg/L	2.5
Calcium (Ammonium nitrate extractable) mg/L	2710
Iron (DPTA extractable) mg/L	21.5
Organic matter %	3.6
Sulphate (phosphate buffer extractable) mg/L	11.0
Manganese (DPTA extractable) mg/L	5.3
Essential Cation Exchange capacity, meq/100g	18.3

Product Application

ACF-32 and ACF-SA were applied by spraying foliarly with high volume of water relative to the standard farm practice in the UK to runoff to reach the soil. The application rate was 3.76 litres per hectare to deliver in 500 litres of de-chlorinated water, using 15 ml of Predator, as recommended by the Nu-trel Group Ltd. Plants were sprayed three times starting from seeding stage to maturity with intervals of around 4-5 weeks using a Berthoud sprayer with yellow Hypro flat fan nozzles x3 at a bar length of 1m applied with a pressure of 2 bar (as recommended). An 18.8ml in 2.5 ml water of product was applied for each 50 m2 plot during 'light rain' conditions to ensure that the soil was wet enough to guarantee good conditions for the bacteria. An extra strip was added to the trial where a high dosage was applied, and measurements were taken as for the other treatments to observe potential phytotoxicity.

Efficacy Measurements

Measurements were devised to ensure appropriate evaluation between treatments for efficacy and included plant height and flag leaf length, chlorophyll content, standard elements in tissue (leaves only), seed nitrogen content, specific weight, protein and yield. Some treatments were performed by supplying samples to third parties certified laboratories in the UK.

Statistical Analysis

The standard analysis of variance was applied to all data Microsoft Excel spread sheet. The results subjected to one-way ANOVA test, Tukey Pairwise Comparisons and Tukey Simultaneous Tests for Differences of Means using.

III. Results and Discussion

Growth Study

The field experiments revealed a significant contribution of ACF-32 and ACF-SA to the growth parameters of barley compared with the control. The results are presented in *Table 2* and *Fig 1* showed variation in measured parameters under different treatments confirming differences. There was a significant effect of applied products on chlorophyll content, plant height, flag leaf length, standard elements in leaves' tissue, nitrogen content, specific weight and yield.

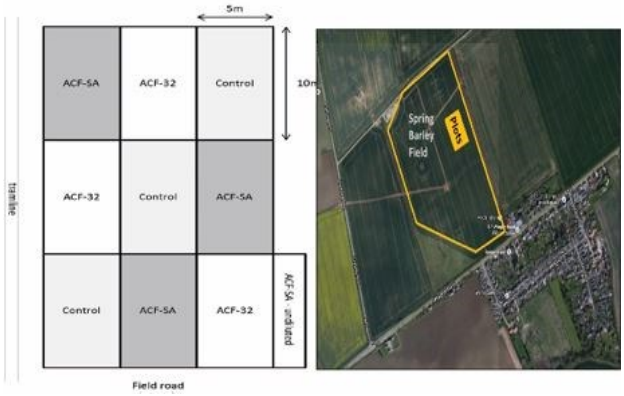

Fig. 1. Experimental Field Design; Position of the trial site in Nettleham, Randomised plot design for spring barley trials.

Chlorophyll concentration was measured twice at different growth stages, and the results are shown in *Fig 2(a)*. The average chlorophyll concentration was lower during the second measurement. However, there was no significant difference between the different treatments during the first ($F(3,196) = 1.95$, $p = 0.122$) and second measurement ($F(2,177) = 1.06$, $p = 0.348$). There was only a trend that plants treated with the pure ACF-SA had lower average chlorophyll concentrations compared to the control and the other treatments during the first measurement as illustrated in *Fig 2 (b)*. Furthermore, there was also a trend that plants treated with ACF-SA at the suggested application rate exhibited higher average chlorophyll concentrations compared to the control and the ACF-32 treatment at both measurements. The previous results can be explained on the basis that the applied treatment of (ACF-SA) is a treatment of (bio-fertiliser) which led to increasing significantly chlorophyll content in barley leaves and any increase in application rate will not benefit towards rising chlorophyll content. This trend was previously illustrated by Abd El-bake (2008) who found that the application of bio-fertilisers increased the total amount of chlorophyll in crops [13].

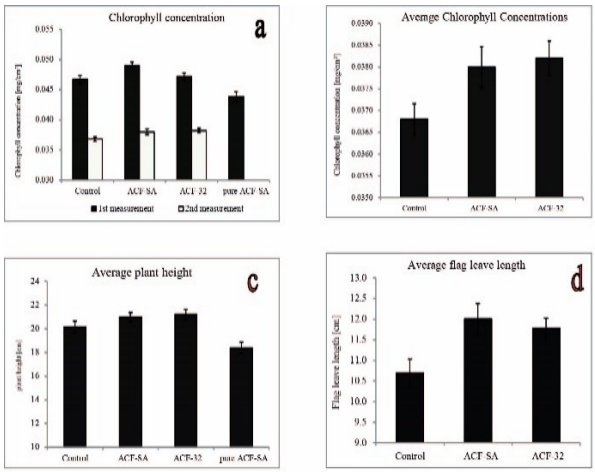

Fig. 2. Measured barley growth parameters; Average chlorophyll concentration [mg/ cm²] at two different growth stages (a), chlorophyll concentration (b), average plant height (c) and average flag leave length (d).

Barley plant average height shown in *Fig 2(c)* confirmed that treatments had no significant effect ($p > 0.05$). Post hoc comparisons using the Turkey HSD test indicated that plants treated with the pure ACF-SA product grew significantly less compared to the ACF-SA ($p = 0.012$) and ACF-32 ($p = 0.005$) treated plants.

Results of the average flag leaf lengths for the different treatments are shown in *Fig 2(d)*. Overall there was a significant difference between treatments (ANOVA, $F(2,87) = 4.53$, $p = 0.013$). Post-hoc comparisons revealed that plants treated with ACF-SA had significantly longer flag leaves compared to the control (Turkey HSD, $p = 0.016$). There was a trend that plants from the ACF-32 treatment had longer leaves compared to the control but not significant (Turkey HSD, $p = 0.058$). There was no difference between the two treatments (Turkey HSD, $p = 0.869$).

Table 2. Tissue analysis results.

Element	Unit	Control	ACF-SA	ACF-32
Nitrogen	% w/w	3.94	4.36	4.28
Phosphorus	% w/w	0.42	0.44	0.38
Potassium	% w/w	2.62	3.77	3.22
Calcium	% w/w	1.35	1.61	1.87
Magnesium	% w/w	0.23	0.15	0.13
Sulphur	mg/kg	3823	3306	4541
Manganese	mg/kg	133	84	82.5
Copper	mg/kg	9.6	12.6	12.8
Zinc	mg/kg	32.7	42.4	41.8
Iron	mg/kg	113	114	231
Boron	mg/kg	6.8	6.4	7.4

Chemical tissue analysis for nitrogen, phosphorous, iron, boron, potassium, calcium, magnesium, sulphur, mangane--se, copper, zinc is demonstrated in Table 2. In general, tissue analysis showed that nitrogen, phosphorous, potassium and zinc where on their higher level in the plants treated with ACF-SA. However, calcium, sulphur, copper, iron and boron were higher in the plants treated with ACF-32. Only magnesium and manganese were higher in the control plants. ACF-SA and ACF-32 treated plants had slightly higher Nitrogen levels compared to the control content of 3.94%, statistically there was no significant difference between the treatments ($p > 0.05$). Potassium content was on its highest levels in both ACF-SA and ACF-32 treated plants compared to the control plants with significant higher level in ACF-SA treated plants of about 3.77%. The average Calcium content in ACF-32 treated plants had the highest levels compared to the control and ACF-SA. Magnesium contents ranged between 0.13-.23 ppm where ACF-SA and ACF-32 treated plants had significantly lower level compared to the control with that of ACF-32 at 0.13 ppm. Interestingly, ACF-32 treated plants had nearly twice the level of Iron compared to the control and ACF-SA.

Harvest – Ears/ Plants/ Yield

Data shown in *Fig 3 (a and b)* demonstrated that there was a significant difference between the treatments (ANOVA, $F(2, 24) = 4.74$, $p = 0.018$) regarding the average number of ears per plant/ per m2. Post-hoc analysis confirmed that ACF-32 treated plants had a significantly higher average number of ears per plant compared to ACF-SA treated plants (Tukey HSD, $p = 0.019$) and the control but not significant (Tukey HSD, $p = 0.083$). Average number of plants per m2 are given in *Fig 3 (c)* showing there was no significant difference and therefore the comparison between treatments is validated (ANOVA, $F(2, 24) = 0.11$, $p = 0.894$).

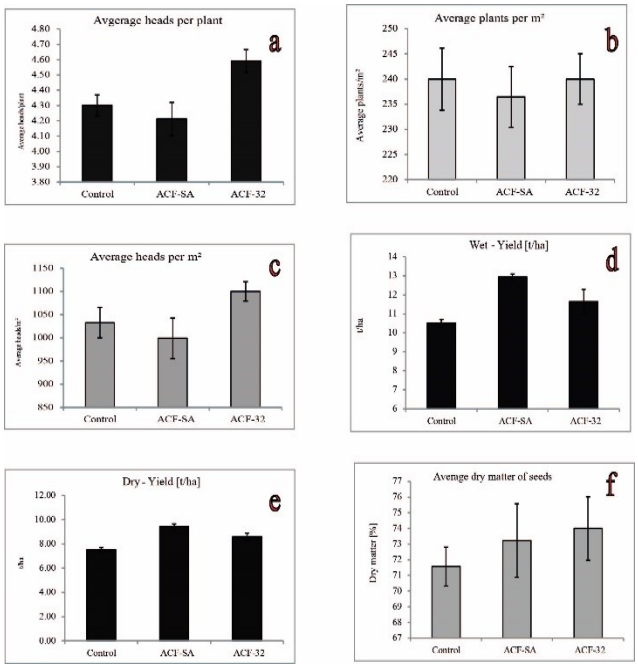

Fig 3. Measured barley yield parameters; average head per plant(a), head per m^2(b), plants per m^2(c), wet-yield t/ha (d), dry yield t/ha (e) and dry matter of seeds (f)

The average yield of the treatments is shown in *Fig 3 (d and e)* for at harvest and dry yield respectively. Overall there was a significant difference between the treatments (ANOVA, $F(2,6) = 6.15$, $p = 0.035$). Post-hoc analysis revealed that plants treated with ACF-SA yielded significantly higher than the control plants (standard farm practice) by 1.94t/ha (Tukey HSD, $p = 0.030$), but not significantly higher compared to ACF-32 treated plants (Tukey HSD, $p = 0.306$). *Fig 3f* showed the average dried matter of seeds after adjustment for moisture as it was confirmed that moisture was not different between treatments ($F(2, 6) = 0.28$, $p = 0.768$). Furthermore, there was a trend of ACF-32 treated plants yielding higher compared to the control but not significant (Tukey HSD, $p = 0.096$).

Further tests for the average grain nitrogen levels in % of dry matter revealed no significant difference between the different treatments and the control (ANOVA, $F(2, 6) = 0.15$, $p = 0.866$) with a great variation between the different plots in the control and the ACF-SA treatment (confidence level of 95%, $p = 0.01$) with values of 1.41, 1.43 and 1.39 % for the control, plants treated with ACF-SA and plants treated with ACF-32 respectively with standard error up to 5%.

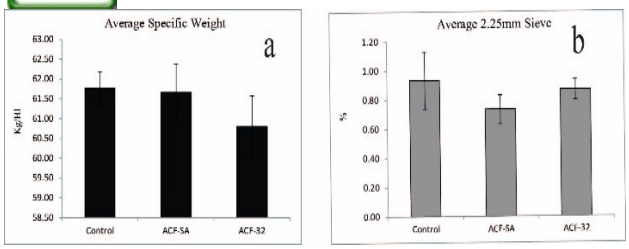

Fig. 4. Measured barley yield parameters; Average specific weight of grain (kg/ hl) depending on the different treatments (a), Average percentage of grain passing a 2.25mm standard slotted sieve (b).

Fig 4, shows the average specific weight of the grains (a) and the average percentage of grains that pass a standard slotted sieve. One-way ANOVA test showed no significant difference between the treatments for both of these parameters. However, there is a trend of ACF-32 treated plants to have a lower specific weight compared to the control and ACF-SA. The obtained results agreed with previously published works concerning the effect of bacteria on the yield and other growth parameters [14-15]

Product dosage and timing will vary depending on soil characteristics, crop choice, application equipment and other agronomic factors. The efficacy of the micro-organisms will vary depending on environmental condition. In particular, moisture and temperature can affect the survival and colonization of the bacteria which is typical in using bacteria cultures to exert their positive effect.

IV. Conclusions

In conclusion, there was a significant difference between the treatment with ACF-SA or ACF-32 and standard farm practice (control) where the treatment of barley with ACF-SA performed better than the control and ACF-32. Yield, which is of most importance to the grower, was higher by 1.94 t/ha compared to standard practice. The protein measurements also demonstrated that ACF-SA produced higher levels compared to the other treatments with lower amount of small grains. ACF-32 although had more ears, it produced lower yield than that of ACF-SA however higher than that of the control. Overall, it was concluded that the treatment ACF-SA was an effective treatment for increasing yield and quality aspects of spring barley.

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