*Preliminary Trial Reports of VBC's Mycorrhizal inocula application*

# Microbial inoculation effects on arbuscular mycorrhizal colonisation, growth and nutrition of wheat and subterranean clover

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## Summary

Commercial inocula of arbuscular mycorrhizal fungi are scarcely available in the market. Research on using commercial inocula are also limited. Therefore, a study on inocula charcaterisation and their effect on mycorrhizal root colonization, growth and nutrition of wheat and subterranean clover was carried out under glasshouse conditions. This study suggests some mechanisms by which inocula influence mycorrhizal symbiosis. However, benefits gained from inoculation of seeds varied because the number of propagules present and their formulation techniques used.

In this study, the effect of AM fungi inculation to subterranean clover and wheat on mycorrhizal root colonisation, nutrition and growth in agricultural soil was significant over no inoculation control treatment. Results of these studies clearly stated that the application of seed inoculation with mycorrhizal inoculum increased plant shoot and root growth at both early and late stages of plant growth all cases over crontol. Influences of arbuscular mycorrhizal inoculation on nutrients uptake varied on quantity of propagules in inoculum. These findings suggested that application of mycorrhizal inoculum can increase nutruients uptake in the subterranean clover and wheat on nutrient-poor sandy soils. But further studies are needed in diferent soil types, various crops and climatic conditions to make greated recommentations.

## Materials and methods

### Experimental design

Sandy soil was collected (0–10 cm) from The University of Western Australia Shenton Park Field Station (31°94′69″S, 115°79′53″E) which had the following chemical properties (see Table 1): 5.3 pH ($CaCl_2$), 3.3 mg $kg^{-1}$ nitrate N, 2.7 mg N $kg^{-1}$ ammonium N, 6.7 mg P $kg^{-1}$ and 27.3 mg K $kg^{-1}$. The collected sandy soil is considered to be nutrients deficient specially phosphorus. Unsterilised soil was used because of the baseline mycorrhizal propogules was few.

Two plants wheat and subterranean clover were chosen as mycorrhizal host plants. Plants seeds were inoculated independtly with three types inoculum namely EndoPrime (10 g per 10 ml of deionised water), EndoMaxx (1g per 10 ml of deionised water) and EndoFuse (1ml per 10 ml deionised water) and grown in pots containing 2 kg of sieved air-dry soil. In addition, each treatment received other essential elements (mg $kg^{-1}$ soil): N (33.3), S (34), Ca (41), Mg (3.95), Mn (3), Zn (2), Cu (0.5), B (0.1), Co (0.1) and Mo (0.08).

There were three plants sown per pot, and the moisture content was maintained at 70% field capacity by frequent checking and watering. Plants were grown in a temperature-controlled glasshouse at The University of Western Australia, Perth (31°58′S, 115°49′E) with the mean day and night temperatures of ~20°C and 15°C, respectively. The study was a randomised complete block design with four replications, and pots were re-randomised within the block periodically to minimise environmental influence. Plants were harvested seven (wheat at end of tillering stage) and ten (wheat at end of anthesis stage) weeks after sowing.

**Plant measurements**

At each harvest, plants were cut at surface level, shoots were placed into a paper bag and roots were separated from soil and washed before placed into paper bag. The plant samples were then oven-dried at 70 ºC for 72 hours for biomass and nutrients analysis. The dried shoots (~200 mg) were digested in a mixture of concentrated $HNO_3$ and $HClO_4$ acids and nutrients' concentration determined by ICP-OES (Optima 5300 DV; Perkin-Elmer Inc., Waltham, MA, USA) as in other studies.

**Mycorrhzal idenfication and DNA sequencing**

Mycorrhizal species were indentified follwing spores morphology. Other fungal and bactrial presence in the inculum was tested by DNA sequencing.

**Assessment of mycorrhizal colonisation**

Roots colonisation by the AM fungi was estimated by the standard procedure. Root subsamples (0.5 g or less fresh weight upon availability) were cleaned by using 10% KOH solution and then stained with trypan blue. Colonisation in the subterranean clover plants was determined as the percentage root length colonised under a dissecting microscope using the gridline intersects method. Gridline intersects were counted for the presence of mycorrhizal structures at ×100 magnification. The counting of mycorrhizal colonisation was carried out in three replicate samples.

**Statistical analyses**

Genstat (v.18) was used to carry out all the statistical analyses. To determine the significant difference among the applied treatments, two-way analysis of variance (crop x inoculation) was used in experiment and Tukey's multiple comparions test was followed to check any significance between the means of the results.

Table 1. Basic properties of wheat straw biochar and soil used in this experiment

| Properties | Soil |
|---|---|
| pH ($H_2O$) | 6.6 |
| pH ($CaCl_2$) | 5.3 |
| EC (dS $m^{-1}$) | 0.03 |
| Carbon (g $kg^{-1}$) | 7.2 |
| Nitrogen (g $kg^{-1}$) | 0.6 |
| C:N ratio | 12.0 |
| $NH_4^+$-N (mg $kg^{-1}$) | 2.7 |
| $NO_3^-$-N (mg $kg^{-1}$) | 3.3 |
| Phosphorus (mg $kg^{-1}$) | 6.7 |
| Potassium (mg $kg^{-1}$) | 27.3 |
| CEC (m.e./100gC) | 3.2 |
| WHC (%) | 25 |

Soil properties where experiment was carried out stated in Table 1 and it is a sandy soil having low nutrients concentations and low mycorrhizal status. Soil pH is near neutral and CEC is very low.

**Results**

**Table 2**. Inoculum properties (claimed by VBC)

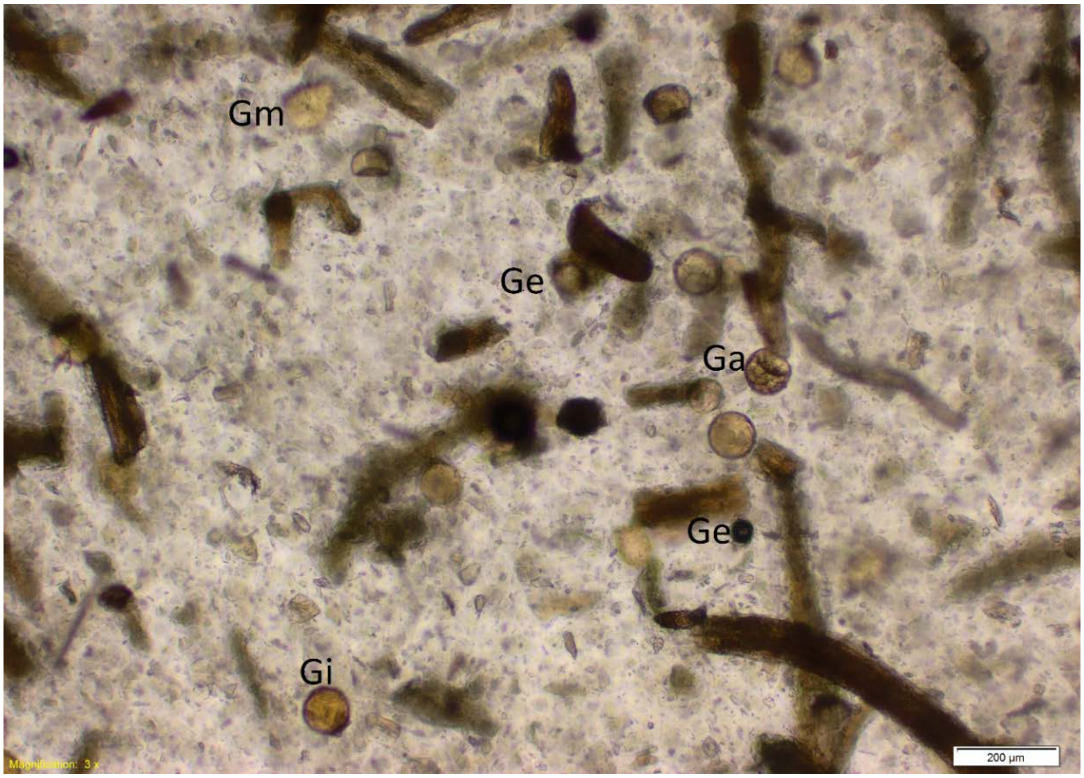
| Inoculum | EndoPrime* | EndoMaxx* | EndoFuse** |
|---|---|---|---|
| Mycorrhizal species (no. g-1) | | | |
| *Glomus intraradices* | 562 | 5620 | 5620 |
| *Glomus aggregatum* | 562 | 5620 | 5620 |
| *Glomus mosseae* | 562 | 5620 | 5620 |
| *Glomus etunicatum* | 562 | 5620 | 5620 |

*contains 15% humic acid derived from leonardite and clay; **other carrier.

**Table 3**. Inoculum properties observed

| Inoculum | EndoPrime | EndoMaxx | EndoFuse |
|---|---|---|---|
| Bactrial gene copy ($g^{-1}$) | 30372 | 25295 | 7 |
| Fungal gene copy ($g^{-1}$) | 45000 | 60332 | 47060 |
| Hyphal length (m $g^{-1}$) | 26 | 40 | 45 |
| Spores (no. $g^{-1}$) | 2030 | 16750 | 20532 |
| *Glomus intraradices* (no. $g^{-1}$) | 510 | 4500 | 5333 |
| *Glomus aggregatum* (no. $g^{-1}$) | 512 | 4200 | 5204 |
| *Glomus mosseae* (no. $g^{-1}$) | 476 | 3716 | 4770 |
| *Glomus etunicatum* (no. $g^{-1}$) | 532 | 4334 | 5225 |
| Gi : Ga : Gm : Ge | 1: 1: 0.9 :1 | 1: 0.9: 0.8: 1 | 1: 1: 0.9: 1 |

Inoculum properties are consistant with VBC's specification but varied bit because of methodolocal limitations.




Morphological identification of spores

Table 4. Other fungal and bacterial species found in the inoculum

| Species | EndoPrime | EndoMaxx | EndoFuse |
|---|---|---|---|
| Fungal species | *Alternaria betae-kenyensis* | *Rhizophlyctis rosea; Epicoccum nigrum; Epicoccum brasiliense; Alternaria betae-kenyensis; Alternaria metachromatica* | *Aspergillus navahoensis; Gautieria monticola.* |
| Bacterial species | *Bacillus: Clostridium; Halobateriales; Terrimonas; Salinimicrobium; Nitrospira* | *Rubrobacter; Salinimicrobium; Pseudomonas; Deinococcus* | ------------ |

Several genus of bacteria and species of fungi were sbserved

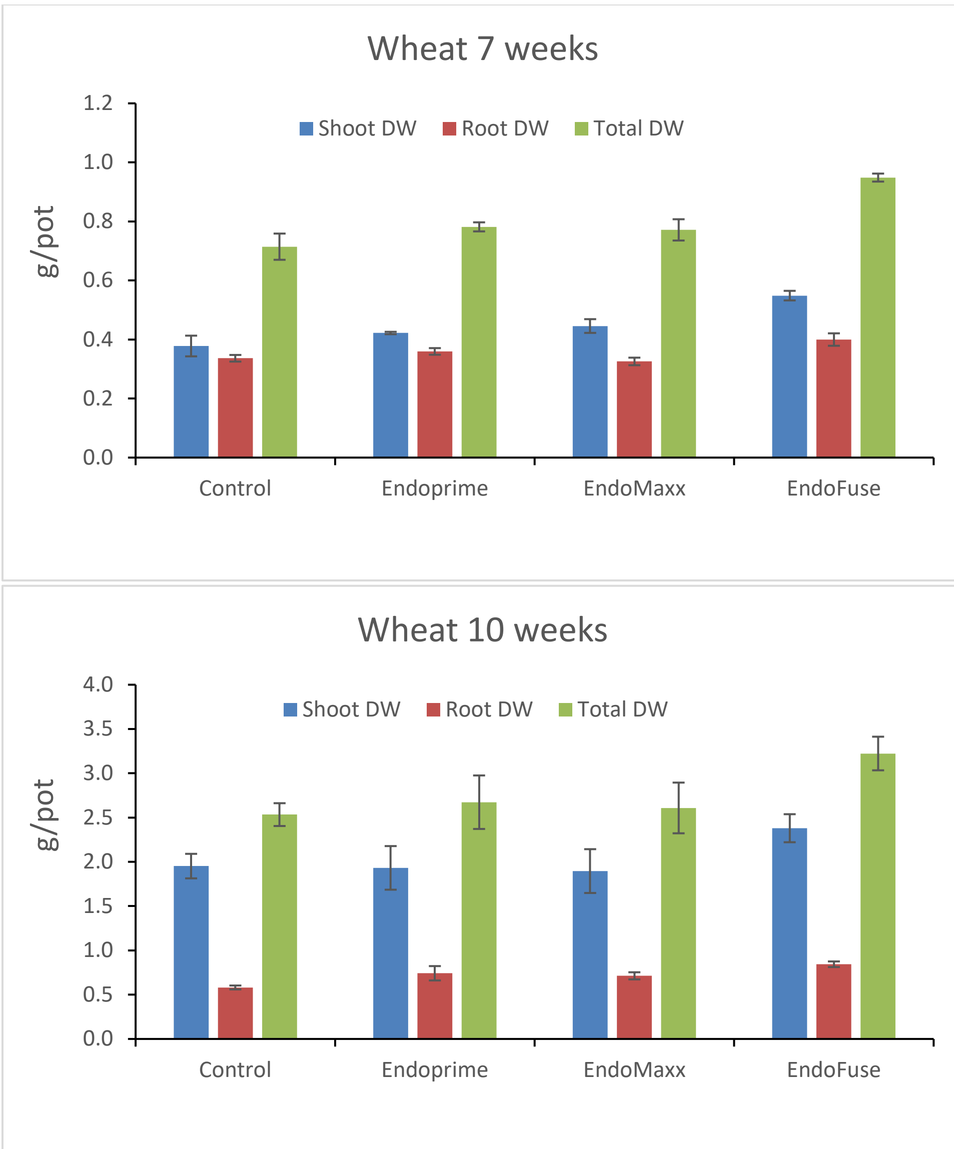
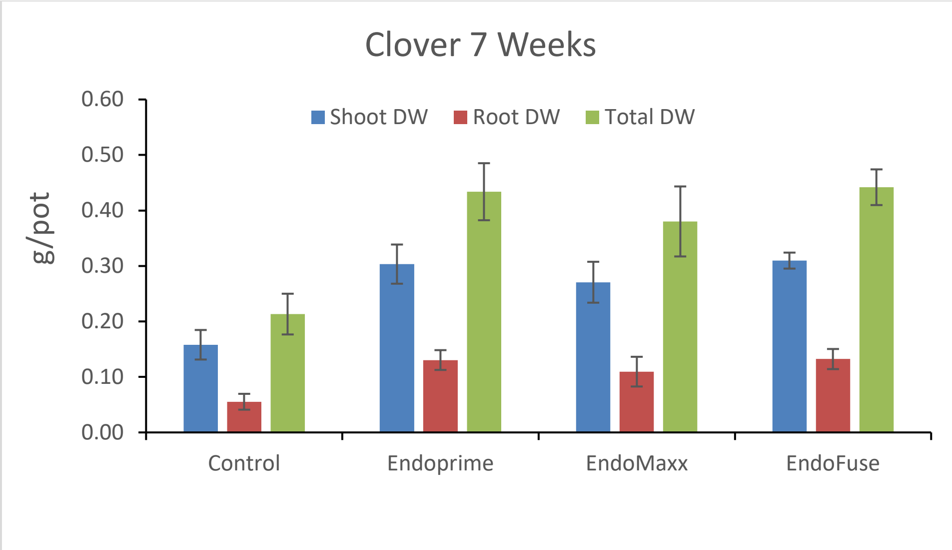



Fig. 1 Effect of inoculum on plant biomass in wheat at 7 and 10 weeks. DW, dry weight.

EndoFuse showed better wheat plant growth on both stages of sampling.



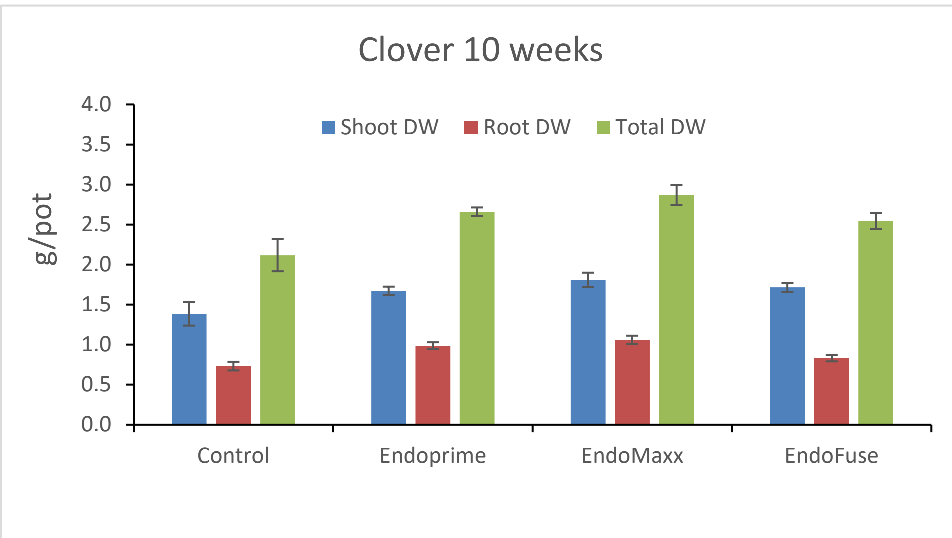




Fig 2. Effect of inoculum on plant biomass in clover at 7 and 10 weeks. DW, dry weight.

Inoculation effect was pronounced in subterranean clover relative to wheat on shoot and root growth.

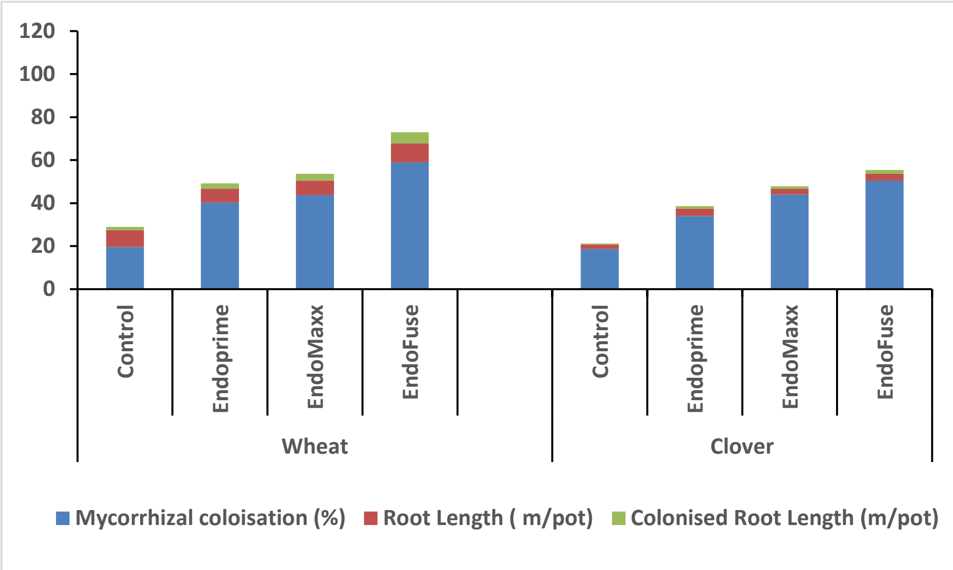



Fig. 3 Mycorrhizal colonisation (%), total root length and mycorrhizal colonised root length at 7 weeks after sowing.

Mycorrhizal colonisation and root traits in all inculum treated crops is higher relative to control.

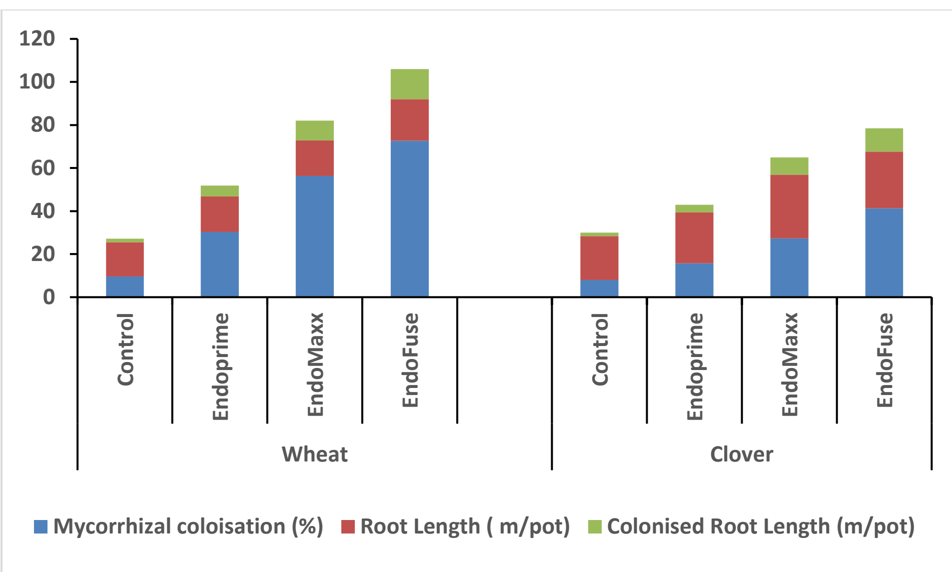



Fig. 4 Mycorrhizal colonisation (%), total root length and mycorrhizal colonised

root length at 10 weeks after sowing.

Mycorrhizal colonisation and root traits in all inculum treated crops are higher relative to control.

Table 5. Nutrients concentration of shoot of wheat and clover at 7 weeks

| Crop | Inoculum | Ca (%) | K (%) | Mg (%) | Na (%) | P (%) | S (%) | Zn (mg/kg) | Fe (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|
| Wheat | Control | 0.20 c | 4.01 a | 0.24 b | 0.02 b | 0.14 a | 0.26 c | 122.3 a | 68.6 c |
| | Endoprime | 0.24 c | 3.78 a | 0.27 b | 0.02 b | 0.16 a | 0.32 bc | 123.2 a | 69.6 c |
| | EndoMaxx | 0.21 c | 4.09 a | 0.25 b | 0.03 b | 0.16 a | 0.27 bc | 117.5 a | 62.7 c |
| | EndoFuse | 0.19 c | 3.91 a | 0.23 b | 0.03 b | 0.14 a | 0.25 c | 100.0 a | 79.4 bc |
| Clover | Control | 1.43 ab | 3.78 a | 0.61 a | 0.70 a | 0.16 a | 0.37 ab | 94.3 a | 107.2 ab |
| | Endoprime | 1.51 a | 3.45 a | 0.59 a | 0.77 a | 0.19 a | 0.34 ab | 113.3 a | 122.7 a |
| | EndoMaxx | 1.45 ab | 3.37 a | 0.56 a | 0.75 a | 0.19 a | 0.39 a | 102.9 a | 105.8 ab |
| | EndoFuse | 1.46 b | 3.34 a | 0.56 a | 0.72 a | 0.19 a | 0.36 ab | 109.8 a | 91.1 abc |

Major nutrients concentrattions did not increased in shoots by mycorrhizal inoculation at 7 weeks of growth.

Table 6. Nutrients concentration of shoot of wheat and clover at 10 weeks

| Crop | Inoculum | Ca (%) | K (%) | Mg (%) | Na (%) | P (%) | S (%) | Zn (mg/kg) | Fe (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|
| Wheat | Control | 0.14 b | 2.06 a | 0.18 b | 0.01 b | 0.51 b | 0.28 b | 52.12 b | 61.65 b |
| | Endoprime | 0.19 b | 1.97 a | 0.22 b | 0.01 b | 0.62 a | 0.31 b | 50.36 b | 76.90 a |
| | EndoMaxx | 0.18 b | 1.74 a | 0.21 b | 0.01 b | 0.45 b | 0.30 b | 78.28 a | 70.58 a |
| | EndoFuse | 0.16 b | 2.19 a | 0.19 b | 0.01 b | 0.60 a | 0.32 b | 51.85 b | 71.55 a |
| Clover | Control | 1.19 a | 1.44 b | 0.52 a | 0.80 a | 0.53 b | 0.82 a | 79.21 a | 88.97 a |
| | Endoprime | 1.16 a | 1.50 b | 0.50 a | 0.78 a | 0.63 a | 0.87 a | 81.50 a | 80.61 a |
| | EndoMaxx | 1.27 a | 1.59 b | 0.55 a | 0.78 a | 0.61 a | 0.81 a | 83.07 a | 87.20 a |
| | EndoFuse | 1.17 a | 1.50 b | 0.52 a | 0.94 a | 0.60 a | 0.81 a | 76.76 a | 83.08 a |

Best indicator of mycorrhizal inoculation effect is an increase of P concentration in shoots observed in both crops.

Table 7. Nutrients uptake of wheat and clover at 7 weeks

| **Crop** | **Inoculum** | **Nutrients uptake (mg/plant)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Ca** | **K** | **Mg** | **Na** | **P** | **S** | **Zn** | **Fe** |
| Wheat | Control | 0.85 e | 16.95 b | 0.99 e | 0.10 c | 0.60 b | 1.09 c | 0.05 a | 0.03 a |
| | Endoprime | 0.95 d | 17.20 b | 1.10 d | 0.08 c | 0.67 ab | 1.36 a | 0.05 a | 0.03 a |
| | EndoMaxx | 0.95 d | 18.19 b | 1.09 d | 0.11 c | 0.73 a | 1.21 b | 0.05 a | 0.03 a |
| | EndoFuse | 1.05 d | 21.47 a | 1.26 c | 0.16 c | 0.77 a | 1.38 a | 0.05 a | 0.04 a |
| Clover | Control | 2.50 bc | 6.57 d | 1.06 d | 1.21 b | 0.28 d | 0.63 d | 0.02 b | 0.02 b |
| | Endoprime | 4.60 a | 10.52 c | 1.78 a | 2.33 a | 0.57 b | 1.04 c | 0.03 b | 0.04 a |
| | EndoMaxx | 3.93 ab | 9.03 c | 1.52 b | 2.00 a | 0.50 bc | 1.04 c | 0.03 b | 0.03 a |
| | EndoFuse | 4.52 a | 10.32 c | 1.74 a | 2.22 a | 0.60 b | 1.12 b | 0.03 b | 0.03 a |

Mycorrhizal inoculation increased major nutrients uptake in both crops relative to control.

Table 8. Nutrients uptake of wheat and clover at 10 weeks

| **Crop** | **Inoculum** | **Nutrients uptake (mg/pot)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Ca** | **K** | **Mg** | **Na** | **P** | **S** | **Zn** | **Fe** |
| Wheat | Control | 2.47 c | 34.82 b | 3.15 d | 0.15 c | 8.91 b | 4.83 c | 0.09 c | 0.11 b |
| | Endoprime | 3.69 b | 38.00 b | 4.21 c | 0.20 b | 12.00 a | 6.02 bc | 0.10 bc | 0.15 ab |
| | EndoMaxx | 3.42 b | 32.00 b | 4.02 c | 0.16 c | 9.00 b | 5.73 bc | 0.14 a | 0.13 ab |
| | EndoFuse | 3.89 b | 52.48 a | 4.44 c | 0.21 b | 14.50 a | 7.56 b | 0.12 ab | 0.17 a |
| Clover | Control | 17.80 a | 22.45 c | 7.72 b | 11.63 a | 7.77 c | 12.90 a | 0.12 ab | 0.13 ab |
| | Endoprime | 19.49 a | 25.17 c | 8.38 ab | 13.01 a | 10.76 b | 14.56 a | 0.14 a | 0.14 ab |
| | EndoMaxx | 23.09 a | 28.42 bc | 9.92 a | 14.22 a | 11.28 b | 14.65 a | 0.15 a | 0.16 ab |
| | EndoFuse | 20.00 a | 25.64 c | 8.83 ab | 15.94 a | 10.26 b | 13.92 a | 0.13 a | 0.14 ab |

Mycorrhizal inoculation increased major nutrients uptake in both crops relative to control.