# IMPROVING PRODUCTION QUALITY OF LILY FLOWERS BY USING ARBUSCULAR MYCORRHIZAL FUNGI AND YEAST AS A VIABLE ALTERNATIVE TO ETHYLENE INHIBITORS

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***Abstract***

The experimental trial was consummated throughout two successive seasons (2010 and 2011) at Ornamental Plants Research and Landscape Dep., Hort. Res. Inst, Giza. It intended to find out the individual effect of mycorrhizal colonization and yeast or the combination between both as a biofertilizer. Besides the combined effect with half dose of NPK as preharvest treatments on ethylene production by flowers and keeping quality of cut flowers of *Lilium asiaticum* cv. Nello. The obtained results showed that all treatments significantly stimulated most of the studied characters compared with that gained from control plants. The inoculation of Lilium with either AM fungi and /or yeast supported the highest values of flower vase life and longevity of flowers and leaves. The addition of AM fungi solely or combined with yeast and/ or ½ NPK gave the maximum flower opening percentage, increased flower diameter, water uptake as well as the percentage of increase in fresh weight. The same combination revealed that leaves had higher total chlorophylls content and the flowers had higher total sugars%. In respect to ethylene production, it was noticed that Mycorrhizal colonization either solely or in combination with yeast and/or ½ NPK decreased flower ethylene production. Thus, mycorrhizal colonization may be a viable alternative to toxic ethylene inhibitors such as silver thiosulfate, since STS has proven to be environmental pollutant.

**Key words:** Vase-life, Ethylene production, Postharvest quality, Arbuscular Mycorrhizal fungi (AMF), Yeast, NPK.

## INTRODUCTION

Flowering bulbs are a group of plants which produce flowers vary in their form, color and seasons. Asiatic lilies have long been produced for use as cut flowers, outdoor (as a flower bed impact and in borders), indoor (as a foliage and flowering pot plants) and have gained popularity in recent years. In Netherlands, Lilium is the second to Tulipa among flower bulbs crop produced (Van der Meulen Muisers, 1999). *Lilium asiaticum* cv. Nello belongs to family "Liliaceae" and nick name is Asiatic lily, is an attractive flowering ornamental bulb, native to America. It has red orange bloom, wide and narrow funnel-shaped, six petalled flowers bloom on long straight stems which decorate the early summer garden like no other plant. The leaves are arranged in whorls around the stem or scattered along it. These flowers are cut off with along

stalk and picking in mature bud stage for facilitating handling, transportation and lowering damage possibilities. Picking can be done also in an earlier stage (when the largest flower bud on each stem show color). The sensitivity of these flowers to ethylene shortens vase life as it stimulates quick opening of flowers which may constitute a big loss to producers and traders. Biofertilizers are commonly named microbial inoculants which are capable of mobilizing important nutritional elements in the soil from nonusable to usable form by plants through their biological processes. Arbuscular Mycorrhizal (AM) fungus can increase plant growth and quality by enhancing water absorption and uptake of nutrients: e.g. phosphorus, potassium and nitrogen as well as Zn, Cu, S, B and Mo., reduces uptake of toxic heavy metals e.g. manganese. It may affect plant water relationships leading to healthy plants. Mycorrhizal colonization has been shown to improve vase life of some cut flowers, but the mechanism is unknown. The possibility exists that prolonged vase life is due to decreased ethylene production. Mycorrhizal colonization may be a viable alternative to toxic ethylene inhibitors such as silver thio sulfate which poses some environmental hazard because of its polluting impact. Active dry yeast is a natural safety biofertilizer. The various positive effects and benefits of applying yeast as a biofertilizer were attributed to its own different nutrients, greater amounts of vitamins and cytokinins as natural plant hormones. In addition, yeast was very effective on releasing carbon dioxide and stimulating photosynthesis (Hashem *et. al*. 2008). NPK are an important source of plant nutrients, however, are expensive and cause environmental pollution.

The present research work aimed to use natural safety environmental materials particularly mycorrhizal fungi for its beneficial effects on reducing floral ethylene production and thus, extending the vase life of cut flowers.

## MATERIALS AND METHODS

This research was carried out at the Experimental green house (seran) of the ornamental plants Res. and Landscape Dep., Horticultural Research Institute, Agricultural Research Center, Giza, Egypt for two successive seasons (2010 and 2011). Bulbs of *Lilium asiaticum* cv. Nello were purchased from a commercial farm with size of 16/18. On 1$^{st}$ January 2010 and 2011, the bulbs were individually planted in 25cm plastic pots filled with 3kg of a mixture of clay: sand: peat (1:1:1,v/v/v).Total microbial count was $5.5x10^{-4}$ $CFUg^{-1}$ soil. Plants were treated as follows during the growth seasons (preharvest):

1- Control.

2- NPK [Krystalon (19-19-19) applied either at the rate of 2g/ pot as full dose or 1g/ pot with other treatments after two weeks from planting and then at two weeks interval during the growth seasons].

3-Yeast (Y) [The pure dry yeast was activated by using sources of carbon and nitrogen with the ratio of 6:1 (Barnett *et. al.*, 1990). It was applied at a concentration of 0.5% twice. The first application was after two weeks from planting and the other was one month later].

4- Arbuscular Mycorrhizal fungi (AMF) [a mixture of mycorrhizal spores was prepared from the rhizosphere by wet sieving and decanting (Gerdemann and Nicolson, 1963). AMF inoculants consisted of 10 ml of wet-sieving suspension of AMF containing 50-60 spores $ml^{-1}$.The application was at planting.

5- Y+ AMF

6- ½ NPK + Y

7- ½ NPK + AMF

8- ½ NPK + Y+AMF

Lily flowers were harvested when the largest flower bud on each stem was showing color and were transported to laboratory of the department then precooling of stems was performed by placing them in an ice cold water for one hours. Stems were recut about 1cm from the spike base, each three flowers were placed in a vase (500 ml) containing 500 ml preservative solution of 2% sucrose and 200 $mg.L^{-1}$ 8- hydroxyquinoline citrate (HQC) under lab conditions of 20 ± 2°C, 50 – 60 % RH and 24 hr light with fluorescent lamps to complete shelf life.

**Measurements**

1-The vase life of cut flowers terminates when the last open flower wilts.

2- Flower longevity determined by the opening of the buds and longevity of flowers.

3- Leaves longevity was defined as number of days in vase life required for 50% of the area became chlorotic or necrotic.

4 - Flower opening percentage.

5- Flower diameter (cm).

6- Water uptake ($cm^3$/spike): was determined by weighing the bottles with and without the flowers every two days.

7- The percentage of increase in fresh weight during vase life.

8- Ethylene production by flowers was assessed each3 days according to the method described by Mayak *et. al.* (1972).

9- Total Chlorophylls in fresh leaves were measured as SPAD reading using 501 Minolta device.

10- Total sugars % in flowers was determined colorimetrically according to the method described by Dubois *et. al.* (1956).

The layout of the experiment was completely randomized design containing 8 treatments. Each treatment was repeated three times, each replicate contained 6 spikes.

**Statistical analysis**

All data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1982) and means were compared by New Less Significant Difference (L.S.D) test at the 5% level of probability in the two seasons.

## RESULTS AND DISCUSSION

**1- Flower vase life and flower longevity (days)**

Lilium characters as affected by Arbuscular Mycorrhizal fungi (AMF), active dry yeast (Y) and mineral fertilization (NPK) and their combination in the two seasons included data of flower vase life and flower longevity (days).The results presented in Table (1) show that all treatments significantly enhanced flower vase life and flower longevity compared with control in both seasons. The highest effect on increasing flower vase life and flower longevity was obtained by the mixture treatments of both microorganisms (Y+AMF) where vase life recorded 17.40 and 17.90 days in both seasons, respectively followed by AMF alone (16.70 and 17.00 days in both seasons, respectively). The results of combination proved that the inoculated treatments by AMF and/or yeast plus NPK recorded significant increase in flower vase life and flower longevity compared with NPK alone in both seasons. These results are in agreement with the findings of a lot of scientists ( Wen (1991), Wen and Chang (1995) on *Gerbera jamesoni* and Besmer and Koide (1999) on *Antirrhinum majus* who revealed that Mycorrhizal colonization significantly increased flower vase-life and the possibility of the prolonged vase-life of cut flowers from mycorrhizal plants is due to decrease ethylene production. Moreover, El-Saka *et al*.,(2002) on amaryllis flowers mentioned that application of 3g/ pot NPK for four times during the cultivation period increased vase life and floret longevity during handling.

**2- Leaves longevity (days)**

The results of Table (1) indicate that both dual inoculants of Y+ AMF (35.40 and 36.00 days, respectively in both seasons) and AMF alone (34.75 and 35.50 days, respectively in both seasons) showed the highest longevity of leaves and they were very effective on preventing leaf chlorosis and abscission completely compared to other treatments used. On the other hand, leaves of control started to show chlorosis beginning from lowermost leaves and recorded the lowest value of flower longevity (24.60 and 26.00 days, respectively in both seasons). Also, it can be noticed that the

inoculation treatments by AMF and/or yeast plus NPK had a marked increase in flower longevity and reduced the occurrence of leaf yellowing compared with NPK alone and the differences were significant in both seasons. These results are in harmony with those of Anushri *et al*., (2002) on Lilium, Scagel (2004) on Harlequin and Besmer and Koide (1999) on *Antirrhinum majus* who stated that Mycorrhizal colonization had a significantly longer vegetative period of leaves.

**3 - Flower opening percentage**

Data exhibited in Table (1) clear that all treatments indicated their superiority over control which had least values of opening percentage (66.67 and 68.40%, respectively in both seasons) with significant differences. The highest values of opening percentage were found in inoculated plants by AMF alone or plus yeast and/or NPK since they attained 100% opening in both seasons. These results are in agreement with Scagel (2004) on Harlequin who pointed out that plants inculated with Arbuscular mycorrhizal fungus opened 7-8 days earlier.

**4- Flower diameter**

The diameter of flower is very important parameter as it is desirable to produce inflorescence with the biggest flowers for using in arrangement. From data in Table (2) it is clear that the greatest flower diameter was obtained from mycorrhizal fungi combined with yeast followed by mycorrhizal fungi alone, whereas the smallest flower diameter was found in control plants for both seasons. Considering the effect of NPK, it can be noticed that NPK combined with mycorrhiza, yeast or the combination of both of them increased the flower diameter compared with NPK alone in both seasons. In this concern the best results were obtained with NPK combined with mycorrhiza and yeast in both seasons. These results are coincided with those of Safwat *et. al*. (2006) on *Euonymus japonicus* and Heikal (2005) on *Thymus vulgaris* who demonstrated that treating the plants with various levels of active dry yeast increased the stem diameter. Also, Anushri *et. al*. (2002) on Lilium and Scagel (2004) on Harlequin stated that mycorrhizal fungi promoted plant development growth.

**5- Water uptake**

Data in Table (2) show differences in water uptake ($cm^3$/spike) of *Lilium asiaticum* cv. Nello when subjected to different treatments. The data cleared that all treatments increased the water uptake which ranged between 165 to 250 in $1^{st}$ season and 170 to 266 $cm^3$ in $2^{nd}$ one more than control (131 and 142 $cm^3$). Yeast combined with AMF followed by AMF alone gave the highest water uptake compared with the other treatments in a significant way in both seasons. Also, the addition NPK to either the combination of Y+AMF or to each of them led to a significant increment

in water uptake compared with NPK alone in both seasons. These results are coincided with those of Besmer and Koide (1999) on *Antirrhinum majus* who revealed that Mycorrhizal colonization improved postharvest quality.

Table 1. Effect of yeast, AM fungi and NPK either single or in combination on flowering & leaves traits of Lilium cv. Nello during two successive seasons (2010&2011).

| **1st season** | | | | |
|---|---|---|---|---|
| **Treatments** | **Vase life (days)** | **Flower longevity (days)** | **Leaves longevity (days)** | **Flower opening (%)** |
| **Control** | **9.10** | **4.60** | **24.60** | **66.67** |
| **NPK** | **11.35** | **5.30** | **29.20** | **73.33** |
| **Yeast (Y)** | **13.0** | **7.80** | **32.80** | **88.89** |
| **AM fungi (AMF)** | **16.70** | **9.00** | **34.75** | **100.00** |
| **Y + AMF** | **17.40** | **9.40** | **35.40** | **100.00** |
| **½ NPK +Y** | **13.50** | **6.00** | **33.50** | **90.98** |
| **½ NPK + AMF** | **14.70** | **7.00** | **34.00** | **100.00** |
| **½ NPK +Y+ AMF** | **15.80** | **7.60** | **34.20** | **100.00** |
| **L.S.D. 0.05** | **2.88** | **2.36** | **2.98** | **3.03** |
| **2nd season** | | | | |
| **Control** | **9.4** | **5.00** | **26.00** | **68.40** |
| **NPK** | **11.60** | **5.70** | **30.0** | **75.00** |
| **Yeast (Y)** | **13.10** | **7.90** | **33.70** | **90.91** |
| **AM fungi (AMF)** | **17.00** | **9.20** | **35.50** | **100.00** |
| **Y + AMF** | **17.90** | **9.60** | **36.00** | **100.00** |
| **½ NPK +Y** | **13.80** | **6.70** | **33.90** | **93.00** |
| **½ NPK + AMF** | **15.60** | **7.50** | **34.20** | **100.00** |
| **½ NPK +Y +AMF** | **16.00** | **8.00** | **35.00** | **100.00** |
| **L.S.D. 0.05** | **2.89** | **2.32** | **3.02** | **3.06** |

### 6- Flower weight increase percentage

The obtained data in Table (2) reveal that the heaviest fresh weights of spikes resulted from application of mycorrhizal fungi combined with yeast or mycorrhizal fungi alone in the two seasons. Control had the least fresh weights of spikes in the two seasons. Considering the effect of NPK, it can be noticed from Table (2) that NPK combined with mycorrhiza, yeast or both of them had a marked increase in the fresh

weight of spikes compared with NPK alone in both seasons. In this regard the better response was occurred with NPK combined with mycorrhiza and yeast in both seasons. The increase in fresh weight more than its initial weight of spike may be associated with an enhancing in water uptake, improving water balance and metabolic activities. Thereby, maintaining the freshness of flowers for a longer period due to enhancing floret longevity and also vase life of spikes. These results are in agreement with Besmer and Koide (1999) on *Antirrhinum majus* and Anushri *et. al*. (2002) on Lilium who showed that mycorrhizal fungi significantly increased shoot fresh weight of the flower. Amer (2004) on *Phaseolus vulgaris* and Heikal (2005) on *Thymus vulgaris* demonstrated that treating the plants with various levels of active dry yeast resulted in an increase in fresh weight of the plants. El-Saka *et. al*. (2002) on amaryllis flowers mentioned that application of 3g/ pot NPK for four times during the cultivation period increased fresh weight during handling.

**7- Ethylene production**

Data in Fig. 1. (A) reflexes that the highest ethylene production was obtained with control followed by nonmycorrhizal plants in both seasons. In these regard all treatments showed a continuous decrease in ethylene production to attain its minimum with AMF alone and yeast+ AMF in both seasons and thus, extends the vase life of cut flowers. Results pointed out that the treatment of inoculation with AMF when combined with yeast and/or NPK showed a positive effect on decreasing ethylene production followed by the treatment of applying NPK combined with AMF or Y compared with NPK alone in both seasons. In contrast, mycorrhizal colonization, treatment with NPK especially phosphor resulted in an increase in ethylene production. These results were shown to be similar to Besmer and Koide (1999) on *Antirrhinum majus* who revealed that Mycorrhizal colonization significantly decreased flower ethylene production. McArthur and Knowles (1992) declared that endogenous ethylene production was significantly reduced in potato roots when colonized by mycorrhizal fungi.

Table 2. Effect of yeast, AM fungi and NPK either single or in combination on flowering traits of Lilium cv. Nello during two successive seasons (2010&2011).

| Treatments | Flower diameter (cm) | Flowers fresh weight increase (%) | Water uptake ($cm^3$/spike) |
|---|---|---|---|
| **1st season** | | | |
| **Control** | **9.9** | **3.53** | **131** |
| **NPK** | **11.62** | **4.45** | **165** |
| **Yeast (Y)** | **12.04** | **5.02** | **195** |
| **AM fungi (AMF)** | **12.68** | **6.20** | **235** |
| **Y + AMF** | **12.89** | **6.90** | **250** |
| **½ NPK +Y** | **12.11** | **5.15** | **207** |
| **½ NPK + AMF** | **12.38** | **5.80** | **221** |
| **½ NPK +Y+ AMF** | **12.40** | **6.14** | **230** |
| **L.S.D. 0.05** | **3.12** | **3.06** | **4.41** |
| **2nd season** | | | |
| **Control** | **10.00** | **4.18** | **142** |
| **NPK** | **11.83** | **4.60** | **170** |
| **Yeast (Y)** | **12.26** | **5.13** | **205** |
| **AM fungi (AMF)** | **12.88** | **6.37** | **250** |
| **Y + AMF** | **12.92** | **7.40** | **266** |
| **½ NPK +Y** | **12.30** | **5.22** | **212** |
| **½ NPK + AMF** | **12.43** | **5.94** | **225** |
| **½ NPK +Y +AMF** | **12.52** | **6.20** | **238** |
| **L.S.D. 0.05** | **3.62** | **2.63** | **3.68** |

## 8- Chemical composition

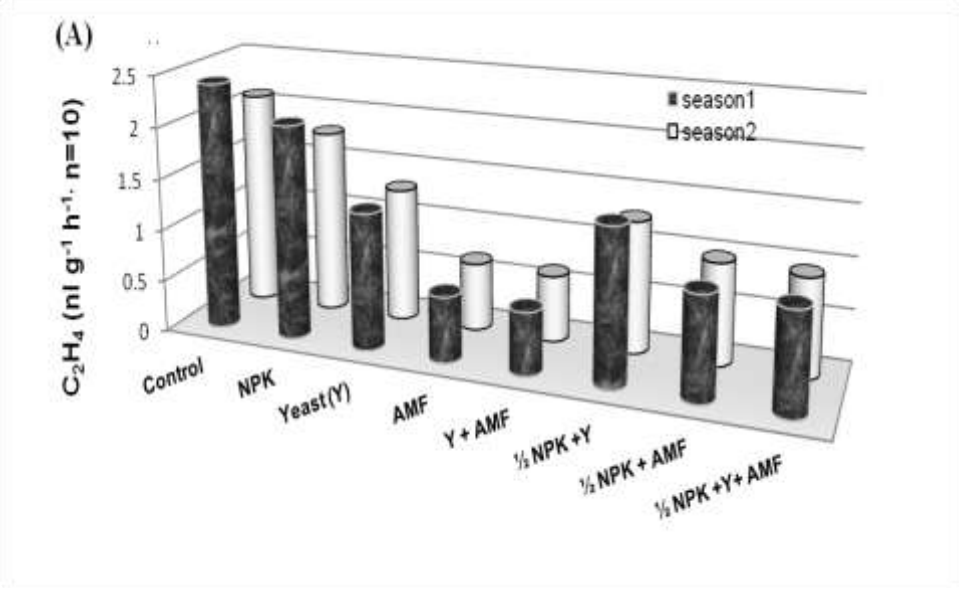
For photosynthetic pigments in fresh leaves: data in Fig. (1B) clear that the greatest chlorophyll (chl) reading was detected in the plants treated with Y+ AMF while the lowest value was recorded with control in both seasons. On the other hand, chl reading in plants treated with NPK alone had lower content of chlorophyll than those plants treated with Y and/or AMF in the presence of NPK in both seasons. The positive effect of yeast on chl content is in harmony with the results of Hayat *et. al.* (2007). Also, Soha and Ezzat (2010) indicated that the plants (*Lupinus termis*) treated

with yeast showed significant increase in chl % in both stages (flowering and vegetative stages). Eissenstat *et. al*. (1990) on sour orange and Lynch *et. al*. (1991) on bean found that mycorrhizal fungi increased photosynthetic activity.

Regarding total sugars % in flowers: it is evident from Fig. (1C) that the inoculation with both biofertilizers exhibited the best influence compared with the control or NPK alone in both seasons. However, the treatment of inoculation with Y and AMF in the presence of NPK gave the highest increase in total sugars % followed by the treatment of applying NPK combined with AMF or Y compared with NPK alone in both seasons. These results were shown to be similar to those of Scagel (2004) on Harlequin who found that mycorrhizal fungi increased carbohydrates by extending the period of photosynthetic activity. The increase in total sugars % as a result of yeast application may be due to the increase in chlorophyll or to enhancing role in cell division and cell elongation producing more leaf area (Hayat, 2007), whereas inoculation with AMF+ yeast enhanced the metabolism process of carbohydrates (Rizk-Alla and Tolba, 2010).

## CONCLUSION

The beneficial effect of the inoculation by mycorrhizal colonization either alone or plus yeast and/or NPK significantly increased flower vase life, improved quality in all examined parameters and significantly decreased ethylene production in both seasons. Thus, mycorrhizal colonization may be a viable alternative to toxic ethylene inhibitors such as silver thiosulfate.



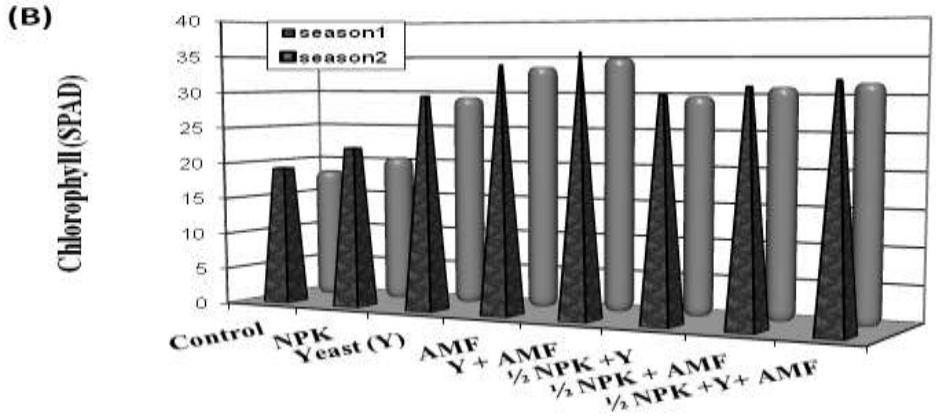




Fig. 1. Effect of yeast, AM fungi and NPK either single or in combination on Ethylene production (A), total chlorophylls in fresh leaves (B) & total sugars in flowers % (C) of Lilium cv. Nello during two successive seasons (2010&2011).

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# تحسين إنتاج ازهار الليليم باستخدام فطرالميكورهيزا والخميرة كبديل حيوي لمثبطات الأيثيلين

**أمال عبد الغفار زكى**

*قسم بحوث نباتات الزينة و تنسيق الحدائق – معهد بحوث البساتين – مركز البحوث الزراعية – الجيزة – مصر.*

أجرى هذا البحث فى الصوبة السيران بمشتل قسم بحوث نباتات الزينة و تنسيق الحدائق – معهد بحوث البساتين – مركز البحوث الزراعية – جيزة – مصر خلال موسمى 2010و 2011 لدراسة استجابة نبات الليليم صنف "نيللو" للتلقيح بفطريات الميكورهيزا الداخلية والخميرة بصورة منفردة أو خليط منهما كسماد حيوى ومقارنته بالتسميد المعدنى. تم قطف الآزهار فى مرحلة البرعم الناضج المغلق ثم نقلها إلى معمل التداول بقسم بحوث الزينة حيث أجريت معاملات مابعد الحصاد.

**وقد أظهرت النتائج الأتى :**

– جميع معاملات التسميد كان لها تأثير منشط لمعظم الصفات النباتية المدروسة مقا رنة بمعاملة الكونترول.

– تلقيح نبات الليليم بفطريات الميكورهيزا الداخلية فى وجود أو عدم وجود الخميرة حققت أعلى زيادة فى عمرالأزهارفى الفازة، وزيادة عمرالزهيرات والآوراق على الساق الزهرية.

– التلقيح المفرد أوالمزدوج بفطريات الميكورهيزا والخميرة وأيضاً إضافة نصف الجرعة الموصى بها من السماد المعدنى أعطت أعلى نسبة تفتح كما أدت إلى زيادة قطر الأزهار وزيادة النسبة المئوية لمعدل الوزن الطازج للأزهار.

– أظهرت النتائج آن إستخدام نفس المعاملة السابقة أدى إلى زيادة محتوى الأوراق من الكلوروفيل كما حققت أعلى زيادة فى النسبة المئوية للسكريات فى الازهار.

– أشارت النتائج إلى أن التلقيح بفطريات الميكورهيزا الداخلية سواء بمفردها أوبإضافتها مع الخميرة فى وجود أو عدم وجود نصف الجرعة الموصى بها من السماد المعدنى أدى إلى تقليل إنتاج الإثيلين من الأزهار.

– وبناء على ما سبق تعتبرالميكورهيزا بديلاً حيوياً أمناً لمثبطات الأيثيلين السامة مثل ثيوكبريتات الفضة حيث ثبت أنه من ملوثات البيئة.