Effect of Four Mycorrhizal Products on Squash Plant Growth and its Effect on Physiological Plant Elements

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Abstract

Vesicular arbuscular mycorrhizal fungi (VAM) are a symbiotic fungi belonging to phylum Glomeromycota, which interact with the root system of higher plants by producing external and internal hyphae, vesiculars and arbuscules. This study aimed to determine the efficiency of VAM fungi in increasing squash plant growth, plant root surface area and its effect on physiological plant elements. Four mycorrhizal products; Bacto_Prof, Endomyk Basic, Endomyk_Conc and Endomyk_Prof were imported from Terrabioscience Company, Germany, contained Glomus intraradices. Each product was used in three doses; half, recommended and double dose according to the application rates and instructions indicated by the manufacturer. Endomyk Basic was the most effective product in improving plant growth. Height of squash plants in the recommended dose treatment was enhanced significantly by 21, 17 and 11%, while P was enhanced by 21, 4, 9 and 8% for Bacto_Prof, Endomyk Basic, Endomyk_Conc and Endomyk_Prof, respectively. Concentrations of fat in shoot was higher than root system, while in crude protein and fiber, the plant root was significantly higher than shoot system by 6 and 52%, respectively. For the carbohydrate concentration, shoot system was higher than root system by 97% increase. From the results of this study, it was concluded that all mycorrhizal products were effective on physiological plant content and plant growth more than non-treated plants.

Keywords: Symbiotic; Glomeromycota; Vesicular arbuscular mycorrhizal fungi

Introduction

Mycorrhiza is a symbiotic association between a fungus and the root system of vascular plants belonging to phylum Glomeromycota [1,2]. Symbiosis termed by De Bary as the mutual beneficial association. Mycorrhizal fungi are the most widespread fungal symbionts that colonize the root system of over 90% of plant species to the mutual benefit of both the plant host and fungus [3,4] either extracellularly as in ectomycorrhizal fungi or intercellularly as in endomycorrhizal fungi [arbuscular mycorrhizal (AM) fungi]. There are different types of fungi that form these symbiotic associations, but for agriculture, the arbuscular mycorrhizal fungi are highly important [2], which colonizes the root system of most cultivated crops and horticultural plants; usually it invades the different layers of the outer root cortex [5].

Vesicular arbuscular mycorrhiza (VAM) colonizes plant roots and extend into the surrounding bulk soil to the root depletion zone around the root system [6]. VAM fungi has no sexual stage so, the only means for gene transfer among individuals is through the vegetative fusion of mycelia [7]. Different crops exhibited different VAM species and different stages of fungal invasion ranging from hyphae, arbuscules and vesicles or combinations of all structures [8].

VAM fungi are associated with improved growth of many plant species due to increased nutrients uptake, production of growth promoting substances, tolerance to drought and salinity and transplant shock and synergistic interaction with other beneficial microorganisms such as nitrogen fixers and phosphorus solubilizers [9]. The major role of VAM fungi is to supply plant roots with phosphorus, because phosphorus is an extremely immobile element in soils [10], due to the fungal extraradical mycelium ability; it grows beside the phosphate depletion zone that quickly develops around the root [3] and can be extended up to 9 cm in the soil [11]. Hyphae of VAM fungi explore a larger volume of soil and phosphate solubilization from unavailable sources present in the soil [12]. Plant phosphate is often the main controlling factor in the plant-fungal relationship [13] and this will be associated with increased plant growth and yield [14].

Therefore, this work was conducted to determine the effect of half of the dose, double of the dose in addition to the recommended application dose of different commercial mycorrhizal products on squash growth and the effect of mycorrhizal products on physiological plant elements.

Materials and Methods

Source of mycorrhizal products

Four different mycorrhizal products were exported from Terrabioscience UG, Bernburg-Germany; Bacto_Prof, Endomyk_Conc, Endomyk_Prof and Endomyk_Basic. Each product was used in different doses according to the application rates and instructions indicated by the manufacturer; Bacto_Prof (1 g/L soil), Endomyk_Conc (1 g/L soil), Endomyk_Prof (2 g/L soil) and Endomyk_Basic (8 g/L soil). All the mycorrhizal products were in powder form except Endomyk_Basic, the mycorrhizae was produced in granule form. We used the recommended double dose.
dose in addition to half dose and double dose for each product to test its effect on plant growth.

**Glasshouse work**

Seedlings of squash plant (*Cucurbita maxima*) var. Yasmina F1 were prepared in trays 2-4 weeks before inoculation with mycorrhizal products. The experiments were carried out under normal environmental conditions 25-32°C under protected cages in the glasshouse, and each pot was irrigated with 100-150 ml tap water day after day.

Mixture of soil: sand (1:2 v/v) ratio was used in this research; soil was sterilized in the oven for 6 hours at 70°C, and then mixed with pure sand in the same day. Seedlings were inoculated with commercial mycorrhizal products and planted in new and clean plastic pots of one liter size and 12 cm depth (Table 1). One fourth liter soil mixture was put at the bottom of the pot then added the mycorrhizal product to the rest amount, to avoid product leaching with irrigation water. The rest of soil mixture with the mycorrhizal products was mixed carefully; then added to the pot around the seedling.

For each crop, five treatments; Bacto_Prof, Endomyk_Conc, Endomyk_Prof, Endomyk_Basic and non inoculated (control) were conducted in three doses in separate experiment. Each treatment was comprised of five replicate pots, grown under glasshouse conditions until harvest time (7-8 weeks). At the harvest day; plants were cut, shoot length was measured, fresh shoot and root weights were recorded. One gram of fine feeder roots from each treatment was taken to examine its mycorrhization according to Philips and Hayman method [15]. Fresh shoots were dried for 24 hrs at 70°C to record its weight. All data were statistically analyzed by using the SAS program, comparison between means was done according to LSD at 5% level.

**Laboratory experiments**

**Evaluation of VAM roots colonization:** Roots were washed carefully to remove soil particles, then heated in 10% KOH at 90°C for half an hour to destroy cell cytoplasm, so the plant cell will be cleared, then washed for 2-3 minutes in running tap water gently, then stained with 0.05% trypan blue in lactophenol according to Philips and Hayman method [15]. Ten root segments with 1 cm length were mounted on each slide and examined microscopically. The incidence and intensity of root colonization with mycorrhiza were calculated using a scale of (0-10) where zero means no colonization, 5 means 50% mycorrhizal root colonization and 10 means 100% mycorrhizal root colonization [16], the readings were taken from the average percentage of thirty roots for each treatment.

**Analysis of plant tissues**

Squash plants inoculated with the recommended dose of mycorrhizal products for 7-8 weeks, were used in analysis of plant tissues, in order to distinguish the differences between the mycorrhizal treated plants and non-treated plants in nutrient absorption; nitrogen, phosphorus, proteins and carbohydrates.

**Determination of moisture:** Samples were dried immediately after harvesting in electrical oven on 70°C for 48 hours then on 103 ± 2°C for 24 hours. To determine the moisture, the samples were in homogenous form, by using the mill with 1 mm sieve under the same conditions. Weight the sample before and after drying to calculate the moisture % and the dry matter % [17].

\[
\text{Calculation: } \% \text{ Moisture} = \left( \frac{A - B}{A} \right) \times 100
\]

| Treatment       | Dose(g/Lsoil) | Height(cm) | FSW(g) | DSW(g) | FRW(g) |
|-----------------|---------------|------------|--------|--------|--------|
| Bacto_Prof      | 0.5           | 29.9       | 10.5   | 2.2    | 4.6    |
|                 | 1             | 31.7       | 11.5   | 2.5    | 4.8    |
|                 | 2             | 33.7       | 13.5   | 3.0    | 5.5    |
| Endomyk_Basic   | 3             | 10.9       | 12.9   | 2.4    | 4.9    |
|                 | 8             | 32          | 13.7   | 2.4    | 5.4    |
|                 | 16            | 35.8        | 14.2   | 3.4    | 6.3    |
| Endomyk_Conc    | 0.5           | 29.9       | 10.9   | 2.4    | 4.9    |
|                 | 1             | 32.2       | 11.6   | 2.6    | 5.5    |
|                 | 2             | 34.7       | 13.5   | 3.0    | 6.3    |
| Endomyk_Prof    | 1             | 30.6       | 10.7   | 2.4    | 4.7    |
|                 | 2             | 30.0       | 10.2   | 2.5    | 4.5    |
|                 | 4             | 33.8       | 13.5   | 3.0    | 5.5    |
| Control         | 0             | 24.1       | 10.6   | 2.2    | 3.4    |

Values are average of five plants, values within each column followed by the same letter are not significantly different (P<0.05) according to LSD.

**Determination of crude fat by ether extracts:** Weight the moisture free sample, clean dry receiving flask was used to place the thimble with the sample, 30-40 ml petroleum solution (organic solution) was added to the receiving flask and placed in sample container then placed under the condenser of the soxhlet apparatus. The extraction lasted for 16 hours, after that the sample was transferred to the oven for drying at 105°C for 30 minutes; then cooled in desicator at room temperature and weighed to calculate the crude fat % [17].

\[
\text{Calculation: } \% \text{ crude fat} = \left( \frac{M_2 - M_1}{M_0} \right) \times 100
\]

Where: M0: original weight of sample

M1: weight of receiving flask before extraction (empty).

M2: weight of receiving flask and fat after extraction.

**Determination of nitrogen and protein by Kjeldahl method:** 0.2 gm dry sample; was prepared by adding 3.50-4.00 gm of the digestion mixture. Put them in a flask, and then add 25 ml sulfuric acid H2SO4, then put it in the digestor. Digest the mixture until the solution becomes colorless, wait for 30 minutes until cool down, then transfer the solution to volumetric flask (100 ml) and complete to 100 ml with distilled water (Table 2).

Place a flask containing 30 ml of 4% boric acid (pH 3.50-4.00) with 3 drops indicator 5:1 (screened methyl red indicator solution) on shelf at the outlet of the condenser of the Kjeldahl distillator. Transfer 5 ml of the filtrated solution to the mixing chamber, and then add 10 ml NaOH 50%, then rinse several times with small amount of distilled water. Free ammonia NH3 from the sample will react with NH4OH and trapped by boric acid to make ammonia biurate with dark blue color between 7-10 minutes. After that, the flask was taken a side for titration, the used volume of HCl must change the blue color to light orange color and this volume of HCl titrated applied to the calculation formula to calculate the N% and protein% [17].

\[
\text{Calculation: } \% N = \left( \frac{\text{vol. HCl sample} - \text{HCl blank}}{\text{No. of HCl}} \right) \times 14.007 \times \frac{100}{1000 \times \text{wt. of sample}}
\]

\[
\text{Calculation: } \% \text{ protein} = \left( \frac{\text{vol. HCl sample} - \text{HCl blank}}{\text{No. of HCl}} \right) \times \frac{100}{1000 \times \text{wt. of sample}}
\]
% protein = % Nitrogen \times 6.25

**Determination of ash:** This method was used to burn off all organic materials and keeping the inorganic materials called ash in order to determine the phosphorus and acid insoluble ash. The crucible used must be clean, so heat it for 1 hour in muffle furnace at 600°C. Cool and weight as quickly as possible. After that weight the sample and place it in muffle furnace on 600°C, leave it for 6-8 hours. After heating, transfer it to the desicator and cool at room temperature, then weight and use the following equation to calculate the ash content:

\[
% \text{Ash} = \frac{\text{wt. of ash (g)}}{\text{wt. of sample (g)}} \times 100
\]

% Organic matter = 100 - % (water + ash)

**Determination of phosphorus (Colorimetric method):** In the crucible with the ash; add 5 ml HCl with 5 drops of nitric acid HNO₃ and dissolve it on hot plate, then filtrate into 200 ml volumetric flask, and dilute it with distilled water. Take 1 ml of the filtered solution to another volumetric flask, dilute it with distilled water, and then add 4 ml ammonium molybdate dissolved in H₂SO₄. Dissolve 0.25 gm of stannous chloride (SnCl₂) in 10 ml of concentrated HCl then add 1 ml for each sample from this solution, then bring up to 100 ml with distilled water. Shake the solution then let it stand for 10 minutes. In this time the color of the solution will turn blue.

Calculate the phosphorus of each sample by measuring the absorbance at 650 nm with spectrophotometer. Distilled water was used as blank, to test the spectrophotometer on zero reading (AOAC, 1995).

\[
\text{% Phosphorus} = \frac{\text{Reading from curve}}{1000000} \times \frac{\text{dilution factor}}{\text{wt. of sample (g)}} \times 100
\]

**Determination of fibers:** Fibers are defined as the organic fraction remaining after digestion with standard solutions of sulfuric acid H₂SO₄ (1.25%) and Sodium hydroxide NaOH (1.25%) under controlled conditions. Weight the filter bag then put in the bag 0.50 gm of dried sample.

Ankom apparatus (01/02) was used in this method. Put the sealed bag inside the Ankom fiber analyzer vessel, add H₂SO₄ 1.25% until it covers the bag, close tightly and turn agitation and heat on for 45 minutes. After that, add NaOH 1.25% until it covers the bag, close tightly for 45 minutes. Then take off the bag, rinse with distilled water, dry the bag in oven at 105°C for 2 hours, then recalibrate the bag weight [17].

**Calculation:**

\[
\text{Calculation: } % \text{Fiber} = \frac{\text{(loss weight} (A-B) \text{gm)}}{\text{(mass of sample gm)}} \times 100
\]

Where: A is weight after extraction process; B is the bag weight empty.

**Results**

**Effect of different commercial mycorrhizal products on squash growth**

Effect of the four mycorrhizal products on squash growth planted with three doses; half dose, recommended dose and double dose was summarized in Table 3. In half dose treatments; there were significant differences in plant height, FSW and FRW. Plant height was improved significantly by all mycorrhizal products over the control plants; Bacto_Prof, Endomyk_Conc and Endomyk_Prof by 11% and Endomyk_Basic by 14%. Plant FSW was increased significantly only in Endomyk_Basic product by 22% more than control treatment. Squash FRW was enhanced significantly in Endomyk_Basic and Endomyk_Conc by 25 and 24% above control plants, respectively.

In the recommended dose; there were significant differences in plant height, FSW and FRW. Height of squash plants was enhanced significantly by all mycorrhizal products; Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof by 17, 18, 19 and 13% in comparison to control plants, respectively. Plant FSW was increased significantly in Endomyk_Basic and Endomyk_Conc products by 38 and 17%, respectively more than control plants, while plant FRW were increased by Bacto_Prof, Endomyk_Basic and Endomyk_Conc products significantly more than control plants; by 43, 58 and 61%, respectively.

In double dose treatments; there were significant differences in plant height, FSW, DSW and FRW. Plant height was increased by all mycorrhizal products more than control plants; Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof by 40, 46, 44 and 40%, respectively. Plant FSW were increased by mycorrhizal products; Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof by 29, 34, 27 and 27%, respectively more than non-treated plants, but there were no differences between the mycorrhizal products in general. The increase in DSW was 37, 57, 43 and 37%, respectively by Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof compared to control plants. However, plant FRW was highly significant in mycorrhizal products compared to non-mycorrhizal plants; by 56, 76, 65 and 61%, respectively for Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof products.

**Evaluations of VAM root colonization**

Mycorrhizal root colonization of the three doses of different products affected squash crop was presented in Table 2. All mycorrhizal products were efficient in root colonization in different intensity. The examination of control plant roots for a possible contamination with mycorrhizal fungi was negative. Endomyk_Basic was more efficient

| Treatment                  | Ncontent (mg/100 gDM) | Pcontent (mg/100 gDM) |
|---------------------------|-----------------------|-----------------------|
| Bacto_Prof (Shoot)        | 1350.4 h              | 145.6 f               |
| Endomyk_Basic (Shoot)     | 1777.6 b              | 139.2 g               |
| Endomyk_Conc (Shoot)      | 1620.5 d              | 139.4 g               |
| Endomyk_Prof (Shoot)      | 1617.6 f              | 139.1 g               |
| Control (Shoot)           | 1254.2 j              | 130.7 h               |
| Bacto_Prof (Root)         | 1578.9 g              | 194.6 a               |
| Endomyk_Basic (Root)      | 1806.5 a              | 167.6 d               |
| Endomyk_Conc (Root)       | 1753.1 c              | 176 b                 |
| Endomyk_Prof (Root)       | 1654 e                | 175 c                 |
| Control (Root)            | 1494 i                | 162 e                 |

Values within each column followed by the same letter are not significantly different (P<0.05) according to LSD.

**Table 3:** Concentration of nitrogen and phosphorus in shoot and root systems of squash plants inoculated with four mycorrhizal products.
than others by 56%. However; the three doses were close to each other in percentage of mycorrhization, and that means this VAM fungi was effective on this crop even in low doses.

**Effect of four mycorrhizal products on physiological plant elements**

Treated plants with mycorrhizal products were highly significant than control in nitrogen and phosphorus content as shown in Table 3. Concentration of nitrogen and phosphorus were markedly higher in root system than in shoot system according to Table 4.

Concentration of nitrogen in shoot system was increased significantly by mycorrhizal products; by 8% for Bacto_Prof, 42% for Endomyk_Basic, 29% for both Endomyk_Conc and Endomyk_Prof more than non-mycorrhizal plants. While in root system, mycorrhizal products increased the concentration of nitrogen by 6, 21, 17 and 11%, respectively for Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof products.

Concentration of phosphorus in shoot system was raised by mycorrhizal products by 11, 6% for Bacto_Prof and Endomyk_Prof, respectively and 7% for Endomyk_Basic and Endomyk_Conc. In root system of squash plant, the concentration of phosphorus was enhanced by 21, 4, 9 and 8%, respectively for Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof products.

There were highly significant differences in ash%, fat%, crude protein%, crude fiber% and carbohydrate% between mycorrhizal products and control plants in all treatments (Table 5). Concentration of ash was higher in control plants than in mycorrhizal products in both shoot and root systems. Concentration of fat was markedly higher in Bacto_Prof followed by Endomyk_Basic and Endomyk_Conc, than Endomyk_Prof in both shoot and root systems. Concentration of crude protein was higher in shoot and root systems by mycorrhizal products; in Endomyk_Basic by 56, 24% followed by Endomyk_Conc by 28, 17% then Endomyk_Prof by 28, 6% and Bacto_Prof by 6, 4% in shoot and root systems, respectively. Concentration of crude fiber was different, where Endomyk_Prof product recorded the highest in shoot, while Bacto_Prof product has recorded the highest in root system. However; the concentration of carbohydrates in shoot and root systems was higher in Endomyk_Basic than all other products by 35, 128% followed by Endomyk_Conc by 43, 80% then Endomyk_Prof 13, 45% and Bacto_Prof by 17, and 22% in shoot and root systems, respectively.

Comparison between plant shoot and root system within each treatment was summarized in Table 6. Concentration of ash in root system was higher than shoot system by 11%, while the concentrations of fat in shoot system was highly increased more than root system. In crude protein and fiber, the plant root system was significantly higher than plant shoot system by 6 and 52%, respectively. For the carbohydrate concentration, shoot system was higher than root system by 97% increase.

**Discussion**

Four mycorrhizal products produced by Terrabioscience Company, Germany, they were effective on different crop plants, in several levels under experimental conditions. The most effective product was Endomyk_Basic in improving plant growth and mycorrhizal root colonization. All contain *Glomus intraradices* with some exceptions of containing other microorganisms such as Bacillus, Algae or other ingredients. Increasing the dose in general enhanced plant growth and increased the root growth by increasing the uptake of water and nutrients.

All mycorrhizal products affected positively on squash crop by producing huge amount of external and internal hyphae, vesicles and arbuscles which will increase the root area surface, thus enhance the growth of the whole plant. Similar results have been reported in Al-Karaki and Al Raddad Al Momany; Al Raddad Al-Momany and Al-Saket [18,19]. The huge network of mycorrhizal hyphae, which spread into the surrounding soil, influence soil fertility and plant nutrition by changing the physico-chemical characteristics of soils stabilizing agents in the formation and maintenance of soil structure [20].

Plant height, shoot fresh weight, shoot dry weight and root fresh weight were significantly different in mycorrhizal than non-mycorrhizal plants in all products. *Glomus intraradices* increased plant yield, height and shoot fresh weight. Mycorrhizal effect is highly dependent on the

![Table 4: Concentration of nitrogen and phosphorus in shoot and root systems of squash plants.](image)

| Treatment          | N %      | P %      |
|--------------------|----------|----------|
| Shoot              | 1523.9 b | 138.8 b  |
| Root               | 1657.3 a | 174.9 a  |

Values within each column followed by the same letter are not significantly different (P<0.05) according to LSD.

![Table 5: Concentration of ash, fat, crude protein, crude fiber and carbohydrates in shoot and root systems of squash plant inoculated with four mycorrhizal products.](image)

| Treatment       | Ash %    | Fat %    | Crude Protein% | Crude Fiber% | CHO %    |
|-----------------|----------|----------|----------------|--------------|----------|
| Shoot           | 31.9 b   | 3.6 a    | 8.4 g          | 22.6 g       | 31.6 c   |
| Root            | 35.5 a   | 1.7 b    | 10.4 a         | 33.3 a       | 16.7 b   |

Values within each column followed by the same letter are not significantly different (P<0.05) according to LSD.

![Table 6: Comparison of the concentration of ash, fat, crude protein, crude fiber and carbohydrates in shoot and root systems of squash plants.](image)
All the mycorrhizal products were effective in affecting physiological plant contents more than in control treatment, except in ash; it was the highest in control plants due to less nutrients present in the dry shoot and root system. Bacto_Prof treated plants contained more fat than other products; however Endomyk_Basic induced more protein and carbohydrates. Concentration of fat and carbohydrates were more in the shoot due to chlorophyll synthesis. While ash, protein and fibers presented in the root were more than in plant shoot. Endomycorrhizal plants contained less carbohydrate than non-mycorrhizal, but roots contained higher content of protein than shoots. The fungus absorbs P from soil and takes carbohydrate from plant cells as a source of energy [22]. Arbuscules are believed to function in bidirectional transfer of nutrients; essentially transfer carbohydrates from plant cell to fungus and minerals especially phosphorus from fungus to host cells [22]. Al Raddad Al Momany [19] recorded that the addition of P fertilizer decreased number of spores on fruit trees, where soil samples were taken from the University farm in Jordan Valley.

Concentration of nitrogen in shoot and root systems of squash were the best in Endomyk_Basic product treatment, while phosphorus showed the best absorption for shoot and root system by Bacto_Prof product. The VAM fungi can increase P uptake in plants that is documented in many researches [9,12,23-25]. P and N uptake were higher in mycorrhizal plants than in control treatment. Plant P is the main controlling factor in the plant-fungal relationship, which plays a significant role in increasing the total uptake of nutrients which leads to the increase in growth and yield [2]. VAM inoculation stimulated the plant growth and it was attributed to enhanced photosynthesis which associated with increased P uptake in leaves stems and flower heads in wheat [18]. Low phosphorus soil showed significant increase in mycorrhizal maize considering P content and total dry weight [26].

Some researchers have indicated that AMF inoculation tends to decrease pH in the rhizosphere, and leads to produce more carbon dioxide (CO₂) [12]. AMF has been assumed to be a major mechanism through increasing carbon inputs to soil and protecting organic carbon from decomposition by aggregation [27]. Many reports have shown that VAM fungi are able to avoid soil erosion by increasing the stability of soil aggregates through the combined action of extraradical hyphae and their exudates. Glomalin is a fungal component, insoluble and hydrophobic proteinaceous substance, which has been reported to improve the stability of soil by avoiding disaggregation by water, so VAM could be used as an indicator of soil and bio-fertilizer in agroecosystems [20,28]. Soil type is a very important factor in the introduction and reproduction of VAM spores, such as clay loam sandy soils which will facilitate the rapid buildup of Glomus populations in rainy areas [8,29].

Conclusions

All mycorrhizal products were effective and significantly different from non-treated plants. VAM fungi increased plant height, fresh shoot weight and fresh root weight in squash crop. Double dose was the most effective treatment, which increased the plant growth and root weight. Endomyk_Basic was the best product in enhancing squash growth. Nitrogen, phosphorus, proteins and carbohydrates were absorbed more by mycorrhizal plants, which enhanced plant growth.

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