Effect of Biofertilizer with Inoculating Fungi on Chili

Thet Su Min[1], Zaw Naing [2], Cho Cho Win[3]

Abstract

Biofertilizer plays a significant role in crop cultivation and broadly applied in organic farming. This study was conducted to investigate the effect of biofertilizer with inoculating fungi (*Trichoderma harzianum*) on growth and yield of chili plant. In the present work, biofertilizer was prepared from farm waste materials such as cow dung, rice straw and rice bran with bioinoculant *Trichoderma harzianum* (Yezin Isolate). All materials such as cow dung, rice straw, rice bran, bioinoculant *T.harzianum* and water were to be used in composting process for preparation of biofertilizer by open heap layering method. Using the compost products mixed with pure soil at different rates, pot experiment was conducted to test the effect of *Trichoderma harzianum* as inoculated compost on the growth of chili plant. The experiment was laid out in Randomized Complete Block Design (RCBD) with four treatments and five replications. Physical parameters and chemical composition of soil samples before sowing and after harvesting were analysed by conventional and modern techniques. Statistical analysis was carried out using International Rice Research Institute (IRRISTAT Version 5.0) in the study. Pot studies observed that 30 % biofertilizer (T3) was able to produce the maximum total yield of 72.62 g plant^{-1} for chili. The inoculated compost (biofertilizer) in this study could thus help reduce the use of chemical fertilizers and promote plant growth and productivity.

Keywords: biofertilizer, *Trichoderma harzianum* (Yezin Isolate), composting process, chili

Introduction

Chili (*Capsicum annuum* L.) is a universal spice widely cultivated throughout temperate, tropical and subtropical countries. It is an indispensable spice due to its pungency, spice, taste, appealing odour and flavor. The dried fruit is extensively used as spices. The ground powder and oleoresin are utilized in pharmaceutical preparations (Warrier, 1989). The chili pepper is a member of the *Solanaceae* family. It is a diploid, facultative, self-pollinating crop, and closely related to potato, tomato, eggplant, tobacco and petunia. They are an important source of nutrients in the human diet and it can be consumed fresh or dried. They promote health benefits such as reducing obesity and diabetes (Shetty *et al*., 2013)

Nutrient management is one of the most important factors to improve the productivity of chilli. The continuous sole and erratic use of chemical fertilizers in imbalance form leads to decline in soil fertility as well as nutrient uptake efficiency of plants, resulting in either yield stagnation or decrease consequently. In last four decades many microorganisms have been used in the form of biofertilizers. Biofertilizers are eco-friendly, low cost input and not only improve the crop growth and yield but also improve fruit quality and fertilizer use efficiency (Patel *et al.,* 2011).

Biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surface, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers can be grouped into four categories:N-fixing biofertilizers, P-solubilizing/ mobilizing biofertilizers, Composting accelerators and Plant-growth – promoting – rhizobacteria. Biofertilizer also includes organic fertilizers (manure, etc.). Use of biofertilizer

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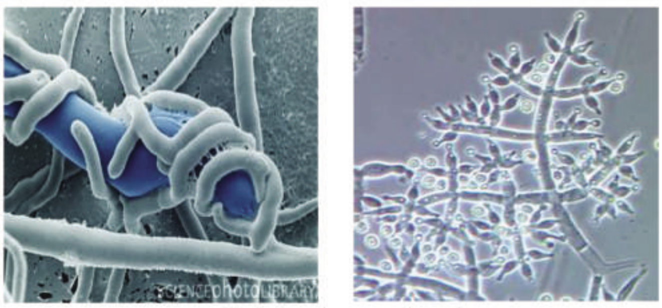
is recommended for improving the soil fertility in organic farming (Vessey, 2003). In recent years, biofertilizers, the products containing living cells of different types of microorganisms, are also used in the integrated nutrient supply system. Biofertilizers can convert nutritionally important elements from unavailable to available form through biological processes leading to crop yields (Hegde *et al*., 1999).

Composting is the transformation of organic material through decomposition into a soil-like material called compost. Invertebrates (insects and earthworms), and microorganisms (bacteria and fungi) help in this transformation. The important advantages of composting are the reduction of the wastes, the destruction of weed seeds and of pathogenic microorganisms. The composting process may significantly reduce the environmental problems associated with the management of manures by transforming them into a safer and more stabilized material for application to soil. The stability and maturity of the compost are essential for its successful application, particularly for composts used in high value horticultural crops (Wang *et al*., 2004).

Trichoderma spp. is a fungal genus found in many regions of the world and widely used because of the multiple beneficial effects on plant growth and disease resistance; in other words it is widely used as biofertilizers and biopesticides (Akladious and Abbas, 2012). *Trichoderma harzianum*, a filamentous fungus is used as a successful biological control agent to control different soil borne plant pathogens. Efficient use of Trichoderma-enriched biofertilizer may increase yield, reduce the uses of N fertilizers, reduce soil borne pathogens and improve soil health. Trichoderma are known as saprophytic mycoparasites which have been effectively cultured on agricultural waste products. (Harman *et al*., 2004).

In Myanmar, many researchers reported the preparation and the use of various kinds of fertilizers. Among them, effect of spirulina on growth yield of onion, tomato and chili (Wai Wai Mar,2007), production of modified fertilizers from bat guano (Theingi Shwe, 2007) and recycling wastes into organic fertilizers by vermicomposting using earthworms (Mi Mi Hlaing, 2011) have been reported. However, this work is very different from research other works. This work is concerned with the effective inoculating fungi mixed with farm waste materials such as cow dung, rice straw and rice bran. The objective of the present study was to investigate the effect of biofertilizer by using *Trichoderma harzianum* as inoculants on yield in chili cultivation under pot experiment.

Figure 1.



Trichoderma harzianum

Material and Methods

Sample Collection and Preparation

Cow dung, rice straw and rice bran were collected from Sein Sar Pin Village, Maeutaw village group, Zaeyarthiri Township, Naypyitaw. All materials such as cow dung, rice straw, rice bran, bioinoculant *T. harzianum* (200: 40: 4: 1) and water (18L) were used in composting process for preparation of biofertilizer by open heap layering method. Size of box used in composting (pile size) was 8' × 4' × 2.5'. After about 75 days, the compost was ready to be used. The fertilizers were packed in bags, stored in a cool place. By using inoculated biofertilizer, the pot experiment was conducted at the Department of Agricultural Research, Yezin. The experiment was laid out in Randomized Complete Block Design (RCBD) with four treatments and five replications. The treatments used in this study were :

Treatment 1 (T1) = Soil treated with 10 % inoculated biofertilizer

Treatment 2 (T2) = Soil treated with 20 % inoculated biofertilizer

Treatment 3 (T3) = Soil treated with 30 % inoculated biofertilizer

Treatment 4 (T4) = control

Weekly plant height measurements were taken from two weeks after transplanting and shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and fruit yield per plant were recorded after the harvest. Land preparation was done by cutting the vegetation of scraping the soil surface.

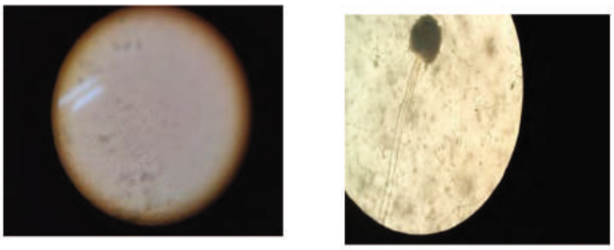
Methods

Physicochemical properties and essential nutrients of prepared biofertilizer were determined by conventional and modern techniques. Microbial identification of biofertilizer was determined by dilution series method. Germination percent of chili seed was evaluated to identify whether complete composting or not. Four treatments were analysed before sowing and after harvesting. Qualitative elemental composition of biofertilizer was determined by EDXRF technique. Measurement of moisture content was determined by oven drying method, pH was carried out by a pH meter and texture was determined by pipette method. Organic carbon was determined by Tyurin's method, electrical conductivity was determined by conductivity meter, available nitrogen content was determined by alkaline permanganate method and available phosphorous content by Olsen's method. Available potassium, exchangeable calcium, magnesium and potassium were determined by AAS. In the analytical procedures of the experiments, recommended methods and techniques were applied (FAO,2008 and AOAC,1980). Statistical analysis was carried out using International Rice Research Institute (IRRISTAT version 5.0) in this study.

Results and Discussion

Population of Microorganism and Maturity of Prepared Biofertilizer

Microbial load in compost was detected for 30 days, 50 days and 70 days. Determination of microorganism from biocompost (after composting) is presented in Figure 2 and Table 1.



(a)*Trichoderma* spp. (b) *Aspergillus niger*

Figure 2. Types of microorganism in biocompost

Initially, the colour of organic matters seems to be brown and turns into black at the end of composting process. The compost was crumbly, loose, and humus-like. It has an earthy smell. The height of compost was decreased gradually during the whole period of composting. After 75 days, the height of biocompost was stable.

Table 1. Determination of Microorganism in Biocompost

Days	Population of Microorganism (CFU)*		
	Trichoderma spp.	*Aspergillus spp.*	*Aspergillus niger*
30	5.0×10^5	6.0×10^5	1.0×10^6
50	3.0×10^6	8.0×10^5	8.0×10^6
70	1.2×10^6	8.0×10^5	1.4×10^6

* CFU – Colony Forming Unit

Figure 3. Maturity of biocompost

Germination percent was evaluated during composting for maturity and stability of compost. Chili seed germination was recorded after 7 days after sowing on peteridish and data were presented in Table 5.

30 days 50 days 70 days

Figure 4. Seed germination for tomato

Table 2. Germination for Chili

Days	Germination (%)
30	40
50	60
70	82

A germination percent higher than 80-85% indicated the absence of phytotoxicity in compost. The good quality of biofertilizer was obtained after composting 75 days. The physicochemical parameters of the biofertilizer were analyzed and the results are shown in Table 3.

Table 3. Physicochemical Properties of Prepared Biofertilizer

Properties	Analytical Value
Moisture (%)	4.28
Bulk Density (g mL^{-1})	0.45
Water Holding Capacity (%)	57.00
pH	7.20
Organic Carbon (%)	18.17
Organic Matter (%)	31.33
C/N ratio	17.47

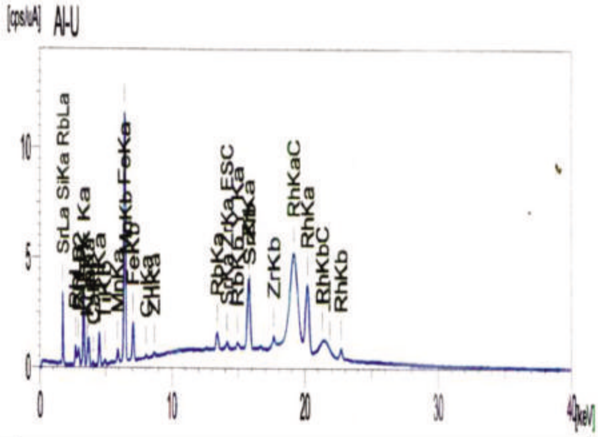
The nutrient contents of the biofertilizer were illustrated in Table 4. The contents of NPK are essential to maintain and sustain the soil fertility. In the present work, the calcium contents were higher than that of magnesium and sulphur in prepared biofertilizer. Iron is necessary for chlorophyll formation. Manganese has several functions in the plant and zinc is one of the most widely used micronutrient.

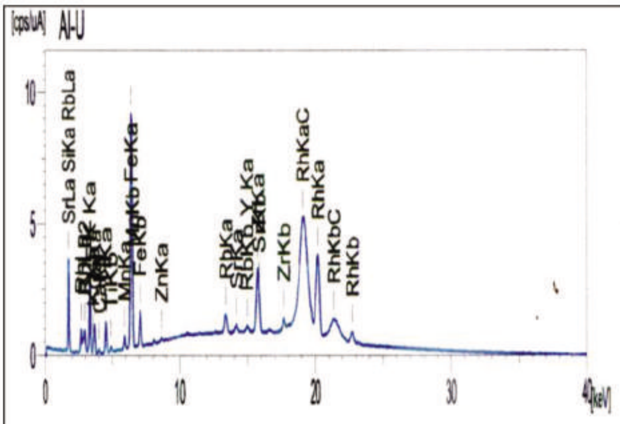
Table 4. Macro and Micronutrients in Prepared Biofertilizer

Macro and Micronutrients	Content (%)
Total N	1.04
Total P	0.20
Total K	0.86
Ca	0.69
Mg	0.15
S	0.05
Fe	0.0908
Mn	0.0074
Zn	0.0098

Elemental Analysis of Prepared Fertilizer by EDXRF

According to EDXRF spectra, essential elements for plants and no toxic elements were found in the four treatments. All the treatments, silicon peaks were the most prominent and so it showed the highest content of silicon.





(T1)

(T2)

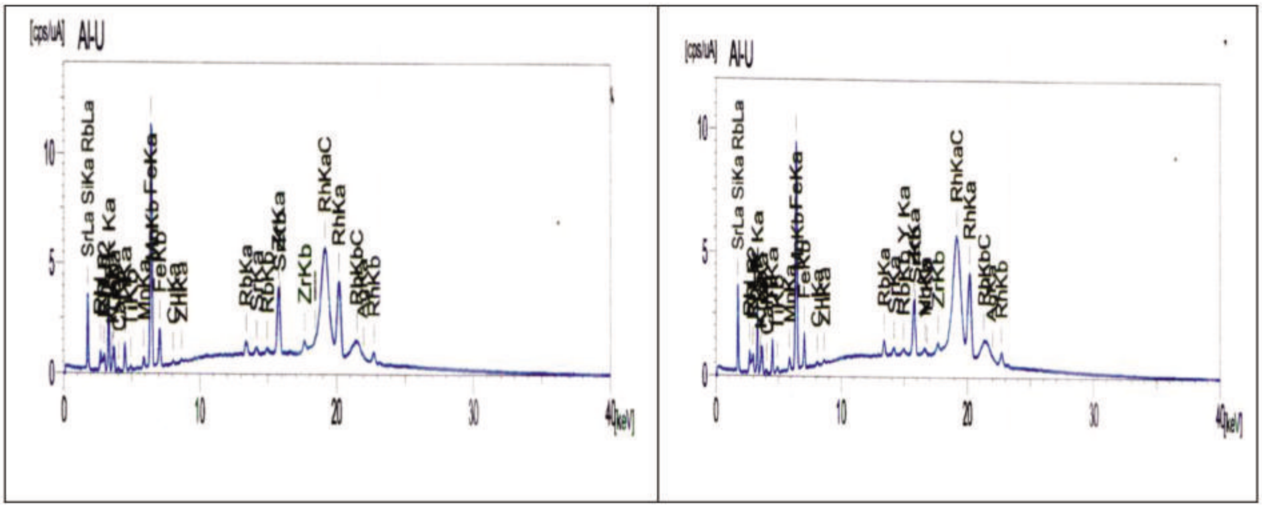


Figure 5. **EDXRF spectra of soil treated with biofertilizer**

Table 5. Elemental Composition of Soil Treated with Biofertilizer by EDXRF

Treatment	T1	T2	T3	T4
Si	74.448	71.612	70.115	74.699
K	12.159	13.517	13.774	11.266
Al	3.914	4.677	4.483	4.474
Ca	3.469	3.816	3.278	3.460
Fe	3.476	3.710	4.018	3.349
Ti	1.409	1.418	1.574	1.480
S	0.380	0.382	0.485	0.377
Mn	0.285	0.294	0.248	0.264
Zr	0.128	0.119	0.107	0.130
Ag	-	-	0.134	0.128
P	0.175	0.245	0.321	0.108
Rb	0.085	0.087	0.071	0.071
Zn	0.024	0.040	0.032	0.033
Cu	-	0.028	0.033	0.032
Sr	0.035	0.033	0.030	0.029
Nb	-	-	-	0.023
Y	0.012	0.015	-	0.011

T1 = 10 % biofertilizer, T2 = 20 % biofertilizer, T3 = 30 % biofertilizer, T4 = Control

Analysis of Soil for Four Treatments before Sowing

Soil is one of the most important natural resources for agricultural production, which heavily depends on soil fertility. It is therefore an essential mandate survey and to classify soil, and to apply fertilizers in order to increase yield. Table 6 shows the physical parameters and chemical compositions of four treatments before sowing. Soil texture depends on the relative proportions of sand, silt and clay in the soil. It indicates high percentage amount of sand in four treatments. This type of soil falls in the domain of loamy sand soil. This type of soil's texture has less capacity to retain water but afford to penetration of roots and being exposed to good aeration and retention of plant nutrients. The pH values of the fertilizer treated soils were found to be slightly increased than that of the original control soil. The pH values of treated soils were found to be in the range of 6.61-6.84. The observed pH values were suitable for plant growth. The values of electrical conductivity of the treated soils were higher than that of the original free soil. Electrical conductivity of soils informs the ionic nature of the soluble compound to supply the needs of plants. Soil humus is also important in increasing the water holding capacity of the soil and it plays a part in the retention of plant nutrients. The humus content increased from 1.79 % to 2.05 % and organic carbon increased from 1.04% to 1.19 %.

As for the total N content in the case of four treatments, T3 has the highest N content. It is a common fact that for plant growth nitrogen (N) is required to promote development of stem and leaf, phosphorous (P) acts to stimulate growth, accelerate fruits and seed formation and the function of potash (or) K is essential to development of starches, sugar and fibers. The highest content of available nitrogen, phosphorous and potassium in T3 were 38.00 ppm, 48.03 ppm and 796.69 ppm and the amounts of exchangeable Ca, Mg and K were about 8.50 me/100g, 0.55 me/100g and 2.68 me/100g respectively. On the context of what has been described above, T4 (control) has lowest N,P and K contents. Regarding the observed values, it is considered that the four treatments can be used in crop production to enhance the soil fertility.

Table 6. Analysis Data of the Soil Before Sowing

Analytical Item	T1	T2	T3	T4
Texture - Sand (%)	83.92	83.80	83.76	83.96
Silt (%)	7.96	7.40	7.84	7.44
Clay **(%)**	8.12	8.80	8.40	8.60
Moisture (%)	1.14	1.44	1.64	0.74
pH	6.66	6.76	6.84	6.61
Electrical Conductivity (dS/m)	0.58	0.86	1.19	0.23
Organic Carbon (%)	1.05	1.15	1.19	1.04
Humus (%)	1.81	1.98	2.05	1.79
Total N (%)	0.11	0.23	0.32	0.06
C/N ratio	9.54	5.00	3.71	17.33
Available N (ppm)	22.00	25.00	38.00	20.00
Available P (ppm)	21.83	39.30	48.03	18.73
Available K (ppm)	350.41	605.79	796.69	272.48
Exchangeable Ca (me/100g)	7.28	7.63	8.50	7.13

Analytical Item	T1	T2	T3	T4
Exchangeable Mg (me/100g)	0.45	0.48	0.55	0.34
Exchangeable K (me/100g)	1.76	1.89	2.68	0.47

T1 = 10 % biofertilizer, T2 = 20 % biofertilizer, T3 = 30 % biofertilizer, T4 = Control

Figure 6. View of pot experiment for chili

Analysis of the Soil used for Chili at Harvesting

Table 7 shows the texture, moisture percent, pH electrical conductivity, organic carbon, humus, Total N, C/N ratio, Available N,P K and exchangeable Ca, Mg, K of soil after harvesting chili plant. The soils were subjected to different treatments by using inoculated compost (biofertilizer). Comparison for all cases is made with respect to the physicochemical composition of the soil samples. After harvesting, all types of soils are found to be loamy sand type.

A variety of treated soils were compared with the control soil after harvesting stage. Moreover, it was observed that the pH of the soil before sowing and after harvesting stages lie between 6.55 and 7.06, plant growth in all experimental pot works. The C/N ratio of T3 is lower than that of other treatments for all crops because large amount of organic fertilizer utilization can cause nitrogen depletion and decrease the C/N ratio.

Number of total fruits of T3 was significantly higher than those of T1, T2 and T4 for all crops. From these results, it was observed that total yield of chili applied with *T.harzianum* were greater than that of these plants without application of *T.harzianum*.

Treatment 1

Treatment 2

Treatment 3

Treatment 4

Figure 7. The growth of chili with prepared biofertilizers

Table 7. Analysis Data of the Soil Using for Chili After Harvesting

Analytical Item	T1	T2	T3	T4
Texture - Sand (%)	86.60	86.20	85.70	87.00
Silt (%)	7.60	7.40	8.40	7.90
Clay (%)	5.70	6.20	5.80	5.00
Moisture (%)	1.20	1.40	1.90	1.10
pH	7.06	6.75	6.95	6.55
Electrical Conductivity (dS/m)	0.15	0.19	0.21	0.14
Organic Carbon (%)	0.90	0.98	1.11	0.83
Humus (%)	1.55	1.68	1.91	1.43
Total N (%)	0.08	0.14	0.17	0.04
C/N ratio	11.25	7.00	6.52	20.75
Available N (ppm)	21.30	23.90	30.40	14.30
Available P (ppm)	41.00	61.00	73.00	36.00
Available K (ppm)	128.00	130.80	154.70	100.50
Exchangeable Ca (me/100g)	2.07	3.20	4.62	0.59
Exchangeable Mg (me/100g)	0.35	0.41	0.32	0.31
Exchangeable K (me/100g)	0.25	0.35	0.40	0.24

T1 = 10 % biofertilizer, T2 = 20 % biofertilizer, T3 = 30 % biofertilizer, T4 = Control

Effect of *T.harzianum* on Growth and Yield of Chili

From the pot experiment investigation, it was observed that *T.harzianum* inoculated biofertilizer promoted plant growth and also enhanced the growth of chili. From the time of sowing to the harvested time was the time frame of 120 days. The growth factors of the crops were evaluated in terms of the plant height (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), number of total fruit per plant and total yield (g $plant^{-1}$).

Plant height and root dry weight were not significant in all treatments ($p>0.05$, Table 8). There was highly significant difference in shoot fresh weight, root fresh weight, shoot dry weight between either treated soil and the original free soil ($p<0.01$, Table 8). A major feature of that *T.harzianum* is its capability to grow along roots during their elongation, thus colonizing the whole root system and benefiting the crop for its entire life (rhizosphere competence). *T.harzianum* has beneficial effects on plant growth and vigour and on the development efficiency of the root systems of several crops (Bjorkman *et al*, 1998).

Number of total fruits of T3 was significantly higher than those of T1, T2 and T4 for all crops. From these results, it was observed that total yield of chili applied with *T.harzianum* were greater than that of these plants without application of *T.harzianum*.

Fruits (T1)

Fruits (T2)

Fruits (T3)

Fruits (T4)

Figure 8. Yield of chili fruits by treated biofertilizers and control

T1 = 10 % biofertilizer, T2 = 20 % biofertilizer, T3 = 30 % biofertilizer, T4 = Control

Table 8. Effect of Biofertilizer on Growth and Yield of Chili

Treatment	Life time (day)	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of total fruit plant^{-1}	Total yield (g plant^{-1})
T1	120	31.94	126.76	12.62	36.78	6.36	36.80	40.92
T2	120	35.60	198.00	12.96	46.73	6.62	43.00	62.13
T3	120	38.86	209.71	15.58	53.31	7.34	53.40	72.62
T4	120	31.94	90.63	10.57	29.57	6.71	25.00	33.29
F-test		ns	**	**	**	ns	**	**
CV%		21.00	4.30	6.30	8.90	9.90	13.10	22.60
LSD(0.05)		10.02	9.20	1.12	5.09	0.92	7.15	16.25

T 1= 10 % Biofertilizer, T 2= 20 %Biofertilizer, T 3= 30 %Biofertilizer, T 4= Control

** = significant ($p<0.01$), ns = non significant

Conclusion

The present research work is conducted to know the effect of *T.harzianum* on plant growth and yield of chili. Based on the specific properties such as total carbon and nitrogen content, the value of macro and micronutrients, pH value and soil texture, EDXRF spectra of prepared biofertilizers and the growth and yield of chili are reliable to enhance the soil fertility and soil productivity. In the present study, it was clearly observed that the *T.harzianum* contained biofertilizer (T3) had positive impact on growth and yield of chili. Agriculture wastes recycling can bring tremendous benefits to agriculture and land management in long run. Therefore, this research may contribute to the development of the biofertilizer for farmers in Myanmar.

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