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RESEARCH ARTICLE

Low cost technology for storage of microbial inoculants

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SUMMARY

The experiment was conducted to store the microbial inoculants such as *Azotobacter chroococcum, Trichoderma viride* and *Pseudomonas fluorescens* in the pitcher technology by using lignite and talc as carrier material. Survivability of these microbial inoculants were monitored upto 120 days. More survival at the end of the 120 days observed in inoculants stored in earthen pot covered with wet sand and least in earthen pot stored at 38^{0}C. Per cent decline survival of *Azotobacter chroococcum* from 0th to 120th day was maximum in inoculants placed in earthen pot maintained at 38^{o}C. Least decline was observed in case of inoculants stored in earthen pot covered with wet sand. Maximum viable cells of *Pseudomonas fluorescens* in lignite at end of storage period was found in earthen pot covered with wet sand. Least number of cells was observed in treatment earthen pot maintained in 38^{o}C. Highest per cent decline was observed in earthen pot maintained in 38^{o}C about 8.8 per cent and lowest of 0.21 per cent in treatment wet sand. Per cent population decreases from 0th day to 120th days of storage of *Trichoderma viride* was more in talc inoculant stored in earthen pot maintained at 38^{o}C followed by carriers at room temperature and inoculants at earthen pot alone. Least per cent population decrease was noticed in inoculants stored in earthen pot covered with wet sand .

Key Words : *Azotobacter chroococcum, Pseudomonas fluorescens,* Storage of microbial inoculant, Pitcher technology

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One of the major constraints in the biofertilizers is lack of consistent field response. There are many reasons for this lack of response in the field. One of the factors contributing to inconsistent field response could be improper storage of these microbial inoculants. Normally the microbial inoculants are stored at ambient conditions until use. Low temperature storage under refrigeration is ideal to enhance / maintain effectiveness and viability. It is not possible for all farmers to store biofertilizer under refrigeration. There is a need to explore possibilities of storing these live microbial inoculants under low temperatures other than refrigeration.

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MATERIALS AND METHODS

Experiment was taken up to test the survival of different microbial inoculants in earthen pots (pitcher) up to 120 days and to assess feasibility of locally available materials in enhancing the shelf life of selected microbial inoculants**.** Lignite and talc based *Azotobacter chroococcum, Trichoderma viride* and *Pseudomonas fluorescens* microbial inoculants were prepared and placed in the pitcher technology.

Establishment of pitcher (earthen pot) technology :

Pitcher technology is an indigenous method to store microbial inoculants. Pitcher technology is nothing but "use of earthen pots for storage of microbial inoculants". Microbial inoculants in two carrier materials were placed in these pots. These pots were placed individually in wooden boxes filled with different locally available materials such as pot kept in wooden box without any filler material served as control, thermocol treatment wooden box was lined inside with thermocol to serve as an insulation material, moist sand, wet paddy straw and saw dust around earthen pot and carrier

material in earthen pot maintained at 38°C. Thermometer was placed into all pots to record the variations of temperature inside the pots and pots were covered with earthen lids. Daily water was sprinkled on surface of filler materials (saw dust, sand and paddy straw) up to saturation point to maintain the moisture content around the pot. Enumeration of *Azotobacter chroococcum, Trichoderma viride* and *Pseudomonas fluorescens* was done by using dilution plate count technique and data obtained were log transformed, statistically analyzed by using Completely Randomized Design (CRD) and means were compared by using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Survival of *Azotobacter chroococcum*, *Pseudomonas fluorescens, Trichoderma viride* in lignite and talc inoculant formulations were monitored for 120 days of storage in earthen pot covered with different locally available materials. Observations were recorded based on the viable cell count taken at 30 day intervals.

Survival of *Azotobacter chroococcum* in lignite and talc stored in different pitcher technology treatments :

In survival study of *Azotobacter chroococcum* more number of viable cells was found in treatment inoculants stored in earthen pot covered with wet sand (log_{10} 7.98 cfu /g). Least was observed in inoculants stored in earthen pot maintained at 38°C (log_{10} 7.12 cfu / g) followed by carriers at room temperature (log_{10} 7.32 cfu / g). Statistically earthen pot covered with wet sand was different than other treatments such as earthen pot covered with saw dust, thermocol and paddy straw. The results obtained are presented in Table 1. In talc as carrier higher survival was found in inoculants stored in earthen pot covered with wet sand (log_{10} 7.98 cfu / g) followed by saw dust (log_{10} 7.82 cfu / g). Significant drop in population was observed in talc inoculant stored at 38°C (log_{10} 7.21 cfu / g) (Table 2).

The rate of per cent decline in population from 0th day to 120 day of storage is presented in Fig 1. Per cent decline survival of *Azotobacter chroococcum* from 0th to 120th day was maximum in inoculants placed in earthen pot maintained at 38°C (13.38 %). Least decline was observed in case of

Table 1: Survival of *Azotobacter chroococcum* population in lignite stored in pitcher technology (log 10 numbers)

Sr.No	Treatments	Population CFU/g lignite (log 10 numbers)					
		Days after storage					
		0	15	30	60	90	120
T_1	Earthen pot	8.22	8.17 ^{d}	8.04 ^{e}	7.99 ^{b}	7.64 ^{c}	7.58 ^{c}
T_2	Earthen pot maintained at 38°C	8.22	8.07 ^{e}	8.00 ^{f}	7.75 ^{c}	7.40 ^{d}	7.12 ^{e}
T_3	Carriers at RT	8.22	8.22 ^{c}	8.28 ^{cd}	8.02 ^{b}	7.45 ^{d}	7.32 ^{d}
T_4	Earthen pot covered with paddy straw	8.22	8.24 ^{bc}	8.29 ^{cd}	8.17 ^{a}	7.98 ^{b}	7.56 ^{c}
T_5	Earthen pot covered with saw dust	8.22	8.26 ^{b}	8.40 ^{b}	8.20 ^{a}	8.11 ^{a}	7.78 ^{b}
T_6	Earthen pot covered with wet sand	8.22	8.30 ^{a}	8.60 ^{a}	8.24 ^{a}	8.15 ^{a}	7.98 ^{a}
T_7	Earthen pot lined with thermocol	8.22	8.26 ^{b}	8.31 ^{c}	8.16 ^{a}	7.96 ^{b}	7.54 ^{c}

Table 2: Survival of *Azotobacter chroococcum* population in talc stored in pitcher technology (log 10 numbers)

Sr. No.	Treatments	Population CFU/g talc (log 10 numbers)					
		Days after storage					
		0	15	30	60	90	120
T_1	Earthen pot	8.18	8.21 ^{b}	8.23 ^{c}	8.05 ^{b}	7.91 ^{c}	7.58 ^{cd}
T_2	Earthen pot maintained at 38°C	8.18	8.13 ^{d}	8.05^{e}	7.80 ^{c}	7.51 ^{f}	7.21 ^{f}
T_3	Carriers at RT	8.18	8.18 ^{c}	8.20 ^{d}	8.02 ^{b}	7.84 ^{e}	7.45 ^{e}
T_4	Earthen pot covered with paddy straw	8.18	8.22 ^{b}	8.22 ^{cd}	8.11 ^{b}	7.90 ^{cd}	7.68 ^{c}
T_5	Earthen pot covered with saw dust	8.18	8.25 ^{a}	8.26 ^{a}	8.23 ^{a}	8.06 ^{b}	7.82 ^{b}
T_6	Earthen pot covered with wet sand	8.18	8.25 ^{a}	8.35 ^{a}	8.26 ^{a}	8.15 ^{a}	7.98 ^{a}
T_7	Earthen pot lined with thermocol	8.18	8.22 ^{b}	8.24 ^{bc}	8.10 ^{b}	7.88 ^{d}	7.56 ^{d}

inoculants stored in earthen pot covered with wet sand (2. 91 %). Per cent decline in the population of *Azotobacter chroococcum* in talc from 0th day to 120th day was observed more in inoculants in earthen pot maintained at 38ºC (11.85 %) followed by carriers at room temperature (8.92 %) and earthen pot alone (7.33 %). Least per cent decline was observed in inoculants stored in earthen pot covered with wet sand (2.44 %) (Fig 2).

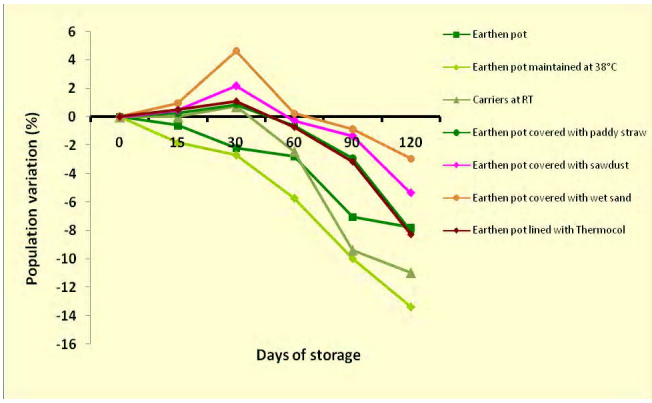

Fig. 1: Per cent survival of *Azotobacter chroococcum* in lignite

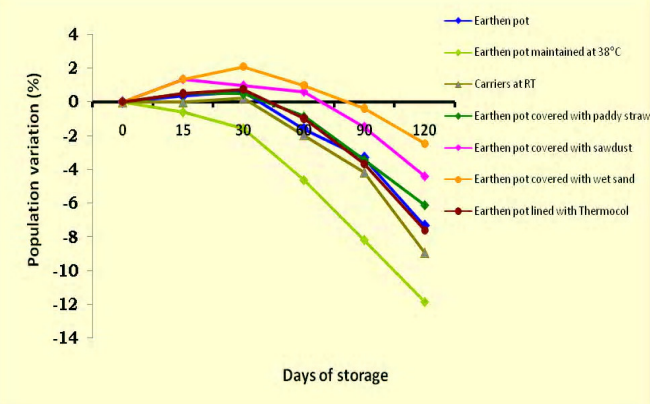

Fig. 2: Per cent survival of *Azotobacter chroococcum* in talc

The inoculants stored in earthen pot covered with wet sand was statistically different than earthen pot covered with thermocol, saw dust and paddy straw. It may be due to moist sand that keeps the earthen pot cool and create a condition of lower temperature in that survival may be high A decline in population on prolonged incubation may be attributed to the depletion of nutrients, moisture content and autolysis of cells (Gaind and Gaur, 1991).

Survival of *Pseudomonas fluorescens* in lignite and talc stored in different pitcher technology treatments :

Maximum viable cells of *Pseudomonas fluorescens* in lignite at end of storage period was found in earthen pot covered with wet sand ($\log_{10}$9.18 cfu / g). Least number of cells was observed in treatment earthen pot maintained in 38ºC ($\log_{10}$ 8.67cfu / g) (Table 3). Highest per cent decline observed in earthen pot maintained in 38ºC about 8.8 per cent and lowest of 0.21 per cent in treatment wet sand (Fig. 3).

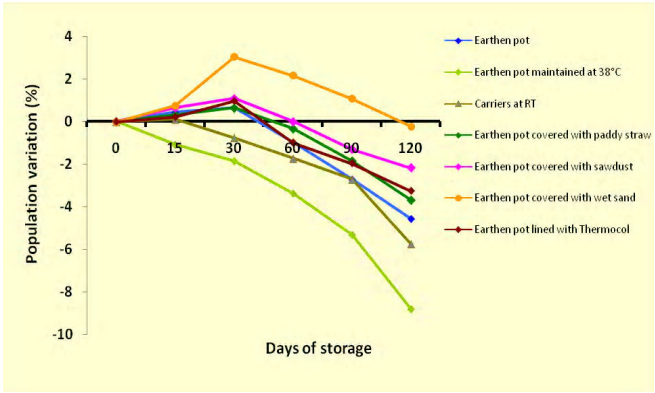

Fig. 3 : Per cent survival of *Pseudomonas fluorescens* in lignite

Survival of *Pseudomonas fluorescens* in talc stored in different substrate in earthen pot was same ($\log_{10}$9.19). At the end of 120th day of storage more number of population was observed in inoculant stored in earthen pot covered with wet

Table 3 : Survival of *Pseudomonas fluorescens* population in lignite stored in pitcher technology (log $_{10}$ numbers)

Sr. No.	Treatments	Population CFU/g lignite (log $_{10}$ numbers) Days after storage					
		0	15	30	60	90	120
T_1	Earthen pot	9.20	9.24 ^{bc}	9.26 ^{b}	9.11 ^{d}	8.95 ^{c}	8.78 ^{d}
T_2	Earthen pot maintained at 38ºC	9.20	9.10 ^{e}	9.03 ^{d}	8.89 ^{f}	8.71 ^{d}	8.39 ^{f}
T_3	Carriers at RT	9.20	9.21 ^{d}	9.13 ^{c}	9.04 ^{e}	8.95 c	8.67 ^{e}
T_4	Earthen pot covered with paddy straw	9.20	9.23 ^{cd}	9.26 ^{b}	9.17 ^{c}	9.03 ^{bc}	8.86 ^{c}
T_5	Earthen pot covered with saw dust	9.20	9.26 ^{ab}	9.30 ^{b}	9.20 ^{b}	9.08 ^{b}	9.00 ^{b}
T_6	Earthen pot covered with wet sand	9.20	9.27 ^{a}	9.48 ^{a}	9.40 ^{a}	9.30 ^{a}	9.18 ^{a}
T_7	Earthen pot lined with thermocol	9.20	9.22 ^{cd}	9.29 ^{b}	9.11 ^{d}	9.02 ^{bc}	8.90 ^{c}

sand (log_{10} 9.17 cfu / g) followed by saw dust (log_{10} 9.11 cfu / g). Least number of viable cells were observed in inoculants stored in earthen pot maintained at 38°C (log_{10} 8.65 cfu / g) followed by carriers at room temperature (log_{10} 8.70 cfu / g) (Table 4). Similar results were found in experiment conducted by Trivedi *et al*. (2005) to study *Pseudomonas fluorescens* viability under storage of 6 months of storage at 4°C and room temperature. Decline in cell number in carrier was greater at room temperature as compared with storage at 4°C.

Per cent decline was more in inoculant kept in earthen pot maintained at 38°C (5.87 %) followed by carriers at room temperature (5.33 %) and inoculants stored in earthen pot alone (4.3 %) (Fig 4).Per cent decline of 0.2 per cent and 5.87 per cent observed in earthen pot covered with wet sand and earthen pot maintained at 38°C, respectively (Fig 4). This might be due to temperature variation and moisture content. Lower temperatures (4-10°C) are known to retard division and metabolic activity of cells resulting reduced consumption of nutrients and reduced loss of moisture in the carriers favouring storage of inoculants. Results obtained are in conformity with the findings of Roughley and Vincent (1967) who have reported optimum moisture levels of 40-50 per cent for maximum survival.

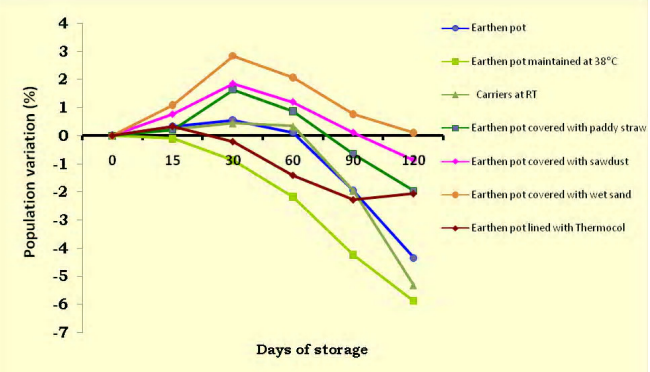

Fig. 4 : Per cent survival of *Pseudomonas fluorescens* in talc

Survival of *Trichoderma viride* population in lignite and talc stored in pitcher technology (log_{10} numbers) :

At the end of 120th day of storage, higher population was found in carrier stored in earthen pot covered with wet sand (log_{10} 7.18 cfu / g) followed by saw dust (log_{10} 7.03 cfu / g), least was observed in inoculants maintained at 38°C in earthen pot (log_{10} 6.35 cfu / g) followed by that kept at room temperature (log_{10} 6.86 cfu /g). The inoculant stored in earthen pot covered with wet sand was statistically significant with the other treatments of storage (Table 5).

Table 4 : Survival of *Pseudomonas fluorescens* population in talc stored in pitcher technology (log_{10} numbers)

Sr. No.	Treatments	Population CFU/g talc (log_{10} numbers) Days after storage					
		0	15	30	60	90	120
T_1	Earthen pot	9.19	9.22 ^{c}	9.24 ^{c}	9.20 ^{d}	9.01 ^{c}	8.79 ^{d}
T_2	Earthen pot maintained at 38°C	9.19	9.18 ^{d}	9.11 ^{d}	8.99 ^{d}	8.80 ^{d}	8.65 ^{e}
T_3	Carriers at RT	9.19	9.21 ^{c}	9.23 ^{c}	9.10 ^{d}	8.99 ^{c}	8.70 ^{e}
T_4	Earthen pot covered with paddy straw	9.19	9.22 ^{c}	9.34 ^{b}	9.27 ^{c}	9.13 ^{b}	9.01 ^{c}
T_5	Earthen pot covered with saw dust	9.19	9.26 ^{b}	9.36 ^{b}	9.30 ^{b}	9.20 ^{ab}	9.11 ^{b}
T_6	Earthen pot covered with wet sand	9.19	9.29 ^{a}	9.45 ^{a}	9.38 ^{a}	9.26 ^{a}	9.17 ^{a}
T_7	Earthen pot lined with thermocol	9.19	9.22 ^{c}	9.32 ^{cd}	9.26 ^{e}	8.98 ^{c}	9.00 ^{c}

Table 5 : Survival of *Trichoderma viride* population in lignite stored in pitcher technology (log_{10} numbers)

Sr. No.	Treatments	Population CFU/g lignite (log_{10} numbers) Days after storage					
		0	15	30	60	90	120
T_1	Earthen pot	7.37	7.35 ^{bcd}	7.36 ^{c}	7.34 ^{ab}	7.21 ^{ab}	6.91 ^{c}
T_2	Earthen pot maintained at 38°C	7.37	7.32 ^{d}	7.28 ^{f}	7.19 ^{c}	6.74 ^{d}	6.35 ^{f}
T_3	Carriers at RT	7.37	7.34 ^{cd}	7.31 ^{de}	7.28 ^{b}	7.18 ^{b}	6.86 ^{e}
T_4	Earthen pot covered with paddy straw	7.37	7.42 ^{abc}	7.41 ^{d}	7.36 ^{ab}	7.08 ^{c}	6.94 ^{d}
T_5	Earthen pot covered with saw dust	7.37	7.45 ^{ab}	7.51 ^{b}	7.39 ^{a}	7.24 ^{ab}	7.03 ^{b}
T_6	Earthen pot covered with wet sand	7.37	7.48 ^{a}	7.62 ^{a}	7.40 ^{a}	7.29 ^{a}	7.18 ^{a}
T_7	Earthen pot lined with thermocol	7.37	7.38 ^{bcd}	7.37 ^{e}	7.34 ^{ab}	7.21 ^{ab}	6.97 ^{c}

Table 6 : Survival of *Trichoderma viride* population in talc stored in pitcher technology (log $_{10}$ numbers)

Sr. No.	Treatments	Population CFU/g talc (log $_{10}$ numbers)					
		Days after storage					
		0	15	30	60	90	120
T_1	Earthen pot	7.41	7.40 ^{bc}	7.39 ^{b}	7.33 ^{c}	7.17 ^{bc}	6.86 ^{c}
T_2	Earthen pot maintained at 38°C	7.41	7.30 ^{e}	7.25 ^{f}	7.09 ^{e}	6.72 ^{d}	6.36 ^{e}
T_3	Carriers at RT	7.41	7.33 ^{d}	7.33 ^{e}	7.27 ^{d}	7.17 ^{c}	6.66 ^{d}
T_4	Earthen pot covered with paddy straw	7.41	7.41 ^{b}	7.40^{cd}	7.37^{b}	7.18^{c}	6.93 ^{d}
T_5	Earthen pot covered with saw dust	7.41	7.42 ^{b}	7.43 ^{bc}	7.40 ^{a}	7.24 ^{ab}	7.04 ^{b}
T_6	Earthen pot covered with wet sand	7.41	7.46 ^{a}	7.48 ^{a}	7.41 ^{a}	7.27 ^{a}	7.20 ^{a}
T_7	Earthen pot lined with thermocol	7.41	7.38 ^{c}	7.41 ^{d}	7.34 ^{c}	7.19 ^{abc}	6.94 ^{c}

The rate of decline in population per cent from 0th day to 120 day of storage is presented in Fig 5. Per cent population decrease in *Trichoderma viride* in lignite was more in inoculant stored in earthen pot maintained at 38^{0}C (13.8 %) followed by that kept at room temperature (6.91 %). Less decline was found in inoculants stored in earthen pot covered with wet sand (2.57 %) followed by earthen pot covered with saw dust (4.61 %). Per cent decline in population from 0th day to 120 day of storage was presented in Fig 5. Per cent population decreases from 0th day to 120th days of storage of *Trichoderma viride* was more in talc inoculant stored in earthen pot maintained at 38^{0}C (14.17 %) followed by carriers at room temperature (10.12 %) and inoculants at earthen pot alone (7.42 %). Least per cent population decrease was noticed in inoculants stored in earthen pot covered with wet sand (2.83 %). These results indicate that refrigerator tend to prolong viability of fungi in this type of formulation. The highest level of population was observed in different time interval (up to 8 months) under refrigerated condition irrespective of the carrier (Papavizas *et al*., 1987).

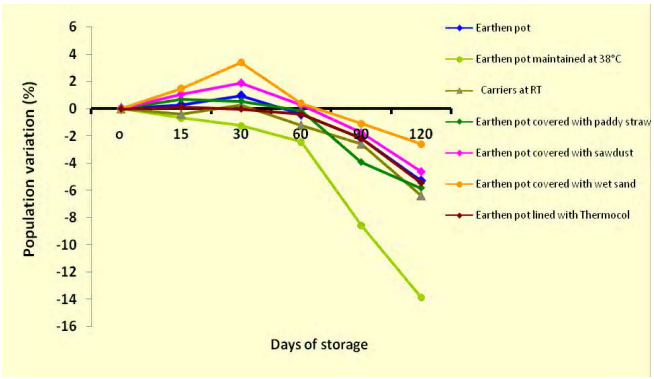

Fig. 5: Per cent survival of *Trichoderma viride* in lignite

Survival of *Trichoderma viride* in lignite and talc was maximum in inoculants stored in earthen pot covered with wet sand (log $_{10}$ 7.18 cfu / g), (log $_{10}$ 7.20 cfu / g) and significant reduction in cells were observed in treatment inoculants stored in earthen pot maintained at 38^{0}C (log $_{10}$ 6.35cfu/g) (log$_{10}$ 6.36 cfu / g), respectively (Table 6). Per cent decline in wet sand was (2.57%). Per cent decline was maximum and observed in T_6 (13.8 %) (Fig. 6). Results obtained at par with survival and proliferation of *Trichoderma* in alginate prills. Survival records obtained in this work indicate that higher survival of viable cells was observed in alginate pellets of *Trichoderma* at 5^{0}C compared to 37^{0}C storage (Lewis and Papavizas, 1985).

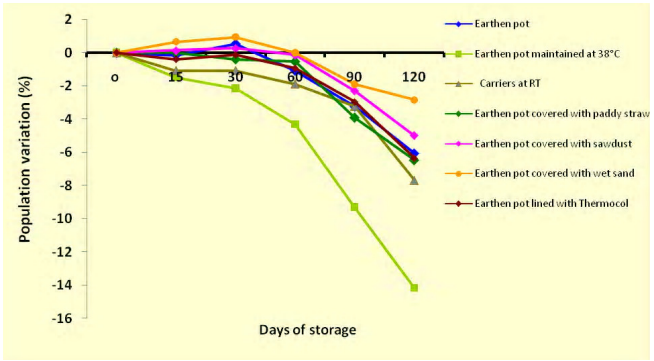

Fig. 6: Per cent survival of *Trichoderma viride* in talc

The result emphasize that for prolonged storage of inoculants, it is necessary to maintain lower temperature irrespective of carriers. Irrespective of carrier / microbial inoculants higher survival rate was observed in treatment earthen pot covered with wet sand and lower survival was observed in case of earthen pot maintained at 38^{0}C.

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