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## Influence of zinc oxide nanoparticles foliar application on zinc uptake of rice (*Oryza sativa* L.) under different establishment methods

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**Abstract**

A field experiment was conducted during *rabi*, 2015-16 and 2016-17 at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad to study the effect of ZnO nanoparticles foliar application on yield and zinc uptake in rice under different establishment methods. The experiment was laid out in split plot design with two establishment methods (direct seeding through hand dibbling under puddled condition and normal transplanting) in main plots and six nutrient management practices (Absolute control-no fertilizer, control-RDF NPK, RDF + soil application of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup>, RDF + two foliar sprays of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 0.5%, RDF + two foliar sprays of nano ZnO @ 1000 ppm and RDF + two foliar sprays of nano ZnO @ 1500 ppm) in sub-plots with four replications. The results revealed that direct seeded rice recorded significantly higher grain and straw yield and total zinc uptake as compared to normal transplanting. Among nutrient management practices, RDF + two foliar sprays of nano ZnO @ 1000 ppm and RDF + two foliar sprays of nano ZnO @ 1500 ppm being on par with each other recorded significantly higher grain and straw yield and total zinc uptake compared to other nutrient management practices.

**Keywords:** Direct seeded rice, normal transplanting, nanoparticles, zinc oxide, zinc uptake

**1. Introduction**

Rice (*Oryza sativa* L.) is one of the most important staple food crop in the world. In Asia, more than two billion people are getting 60-70 per cent of their energy requirement from rice and its derived products. Total geographical area under rice in India is 42.96 million hectares with annual production of 112.91 million tonnes with productivity 2.63 tonnes ha<sup>-1</sup> (DoES, 2018) [1].

Imminent water crisis, water demanding nature of traditionally cultivated transplanted rice and climbing labour costs rattle the search for alternative management methods to increase water productivity, system sustainability and profitability. Direct seeded rice (DSR) technique is becoming popular now a days because of its low-input demanding nature. It offers a very exciting opportunity to improve water and environmental sustainability (Bouman, 2001) [1]. Under the DSR technology sowing of rice seeds is done directly in the soil rather than transplanting seedlings. The reduced emission of these gases helps in climate change adaptation and mitigation, enhanced nutrient relations, organic matter turnovers, carbon sequestration and also provides the opportunity of crop intensification

The productivity of rice soil depends upon the adequate and balanced nutrition of all essential nutrients including the micronutrients. In rice, continuous use of NPK fertilizer has remarkably increased production but simultaneously brought about problems related to micronutrient deficiencies, particularly that of zinc in soil.

In most cases, rice cultivated soils are very low in plant-available Zn leading to further decreases in grain Zn concentration (Cakmak, 2008) [3]. Previous studies have reported that about 30% of the cultivated soils of the world are Zn deficient and about 50% of the soils used for cereal crop production have low levels of Zn available for plants (Cakmak, 2002) [2].

Zinc (Zn) is one of the important micronutrient required for plant growth and development. Soil Zn deficiency is a major problem and hence adequate Zn supply during crop developmental stages are recommended to improve the nutrient content in the rice grain and

also improve productivity of the soil. Crops require only small amount of Zn for their normal growth but its application rate is high due to very low fertilizer use efficiency which vary 1-3 and 5-8% in soil and foliar application, respectively. Hence, there is a need to have formulations of Zn with improved use efficiency for the better crop performance with less input.

Nano fertilizers are synthesized in order to regulate the release of nutrients depending on the requirements of the crops and it is also reported that nano fertilizers are more efficient than ordinary fertilizer (Suman *et al.*, 2010) [13]. In recent past, the positive effect of nanofertilizers on germination, photosynthesis, growth, nutrient absorption, fertilizer use efficiency and yield have been studied in various crops under pot culture and field studies. Keeping these points in consideration, the experiment was carried out to study the effect of zinc oxide nano particles foliar application on zinc uptake of rice (*Oryza sativa* L.) under different crop establishment methods.

### Material and Methods

A field experiment was conducted during *rabi* 2015-16 and 2016-17 at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad. The initial soil parameters were pH=8.1; EC= 0.28 dS m<sup>-1</sup>; organic carbon = 0.46%; available nitrogen = 229.0 kg ha<sup>-1</sup>; available P = 21.9 kg ha<sup>-1</sup>; available K= 399.0 kg ha<sup>-1</sup>; available zinc = 0.68 and available iron= 6.40 ppm. The analysis of soil sample revealed that soil was clay loam in texture having low organic carbon and available nitrogen, medium in available phosphorus and high in available potassium contents with moderately alkaline in reaction. The available zinc and iron content were sufficient. Rice variety varadhan (DRR Dhan 36) is tested in the experiment.

The experiment was laid out in split plot design with two crop establishment methods (DSR-direct seeding through hand dibbling under puddled condition and NTP- normal transplanting) in main plots and six nutrient management practices (Absolute control-no fertilizer, control-RDF NPK, RDF + soil application of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup>, RDF + two foliar sprays of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 0.5%, RDF + two foliar sprays of nano ZnO @ 1000 ppm and RDF + two foliar sprays of nano ZnO @ 1500 ppm) in sub-plots with four replications. The recommended dose of fertilizer (RDF) for rice is 120, 60 and 40 kg N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O ha<sup>-1</sup> respectively. Nitrogen was applied in three equal split doses i.e. basal, 45 DAS and 75 DAS in direct seeded rice and basal, 30 DAT and 60 DAT under normal transplanting, respectively. Full dose of P, K and Soil Zn were applied as basal dose. The nutrients N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were supplied through urea, single super phosphate and muriate of potash, respectively. The foliar applications of zinc were carried out at two stages i.e. 15 DAT and 25 DAT in transplanted rice and 40 DAS and 50 DAS in direct seeded rice through knapsack sprayer. Equal spacing of 20 cm x 15 cm was adopted in direct seeding and normal transplanting. For transplanting, 25 days old seedlings were used. The soil kept at saturation level throughout crop growth period. For preparation of particle suspension, ZnO-nanoparticles were suspended in the deionized water directly and dispersed by ultrasonic vibration for 30 min. The aggregation of particles was avoided by stirring the suspensions with magnetic bars.

At harvest, plant samples from each plots were harvested to record the grain and straw yield. For chemical analysis of zinc content, plant samples were oven dried and ground into fine powder. Zinc content was determined after digesting the plant

material with tri-acid mixture of 9:4:1 (HNO<sub>3</sub>: H<sub>2</sub>SO<sub>4</sub>: HClO<sub>4</sub>) (Piper, 1966) [9]. Zn content was determined by using atomic absorption spectrophotometry (David, 1960) [4]. The Zn uptake in rice was calculated by multiplying zinc content with the respective plant dry weight. The Zn content of grain and straw was analyzed separately and then multiplied with respective grain and straw yields to present the Zn uptake at harvest and expressed in g ha<sup>-1</sup>.

The data recorded on various parameters of the crop during the course of investigation was statistically analyzed following the analysis of variance for split plot design given by Gomez and Gomez (1984) [6].

## Results and Discussion

### Grain, straw and biological yield

The mean grain yield was 4965 kg ha<sup>-1</sup> in 2015, 4688 kg ha<sup>-1</sup> in 2016 and 4829 kg ha<sup>-1</sup> in pooled mean. In spite of the treatment differences, higher grain yield was recorded during *rabi* 2015 than *rabi* 2016 and it may be attributed to congenial weather parameters (rainfall, solar radiation and temperature) and yield attributes during first year (Table 1).

Direct seeded rice (DSR) recorded significantly higher grain yield (5198, 4886 and 5047 kg ha<sup>-1</sup>) over normal transplanted rice (4733, 4489 and 4611 kg ha<sup>-1</sup>) during both the years and in pooled mean, respectively. The per cent grain yield increase in DSR was 9.84, 8.85 and 9.45 % over normal transplanted rice (NTP) during 2015, 2016 and their pooled mean, respectively. Higher grain yield under DSR is mainly attributed to significantly more number of panicles per unit area under DSR compared to NTP.

The more panicles m<sup>-2</sup> in DSR may be attributed to higher number of tillers per hill and vigorous plant growth than the transplanted rice. The robust plants under DSR could be due to the deeper and vigorous root growth which enabled them to access nutrients from much greater volume of soil. Crop establishment through direct seeding avoids transplanting shock to the seedlings at initial stage unlike normal transplanting where growth disruption occur due to transplantation shock. Direct seeding under puddled condition provides better opportunity for establishment, deeper and vigorous root system, robust plant growth, more leaf area and LAI, more chlorophyll content, higher light harnessing capacity, more number of tillers and panicles per unit area (Peng *et al.*, 2006) [8]. While reviewing available literature it was found that poor yield under DSR is mainly caused due to poor or uneven plant population (Rickman *et al.*, 2001) [11], heavy weed infestation (Kumar *et al.*, 2008) [7] and improper water and nutrient management practices (Yadav *et al.*, 2011) [15]. But in present study, DSR is sown through manual hand dibbling at optimum depth under puddled condition which resulted in good germination and uniform plant population at desired spacing which in turn facilitated manual weed control. Straw yield was also significantly higher under DSR (6675, 6447 and 6561 kg ha<sup>-1</sup> during 2015, 2016 and pooled mean, respectively) compared to normal transplanting. Increase in straw yield was in the tune of 11.3, 10.9 and 11.1% under DSR over NTP during both the years and pooled mean, respectively (Table 1). This might be due to more number of tiller per unit area and higher dry matter production in DSR.

RDF+ 2 FS of nano Zn @ 1000 ppm (N<sub>5</sub>) was found to give significantly higher grain yield during both the years of study over other nutrient management practices except RDF+ 2 FS of nano Zn @ 1500 ppm (N<sub>6</sub>) (Table 1). The grain yield of rice in RDF+ 2 FS of nano Zn @ 1000 ppm (6070, 5659 and 5878 kg ha<sup>-1</sup>) and RDF+ 2 FS of nano Zn @ 1500 ppm (5978,

5574 and 5776 kg ha<sup>-1</sup>) were statistically at par with each other but these nutrient management practices were significantly superior over absolute control (2335, 2274 and 2305 kg ha<sup>-1</sup>), RDF (4739, 4469 and 4604 kg ha<sup>-1</sup>), RDF+ SA of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (5187, 4993 and 5090 kg ha<sup>-1</sup>) and RDF+ 2 FS of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 0.5% (5483, 5157 and 5320 kg ha<sup>-1</sup>) during 2015, 2016 and pooled mean respectively. The per cent increase in pooled grain yield was 10.54, 15.54, 25.44 and 27.65% in RDF+ SA of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (N<sub>3</sub>), RDF+ 2 FS of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 0.5% (N<sub>4</sub>), RDF+ 2 FS of nano Zn @ 1500 ppm (N<sub>6</sub>) and RDF+ 2 FS of nano Zn @ 1000 ppm (N<sub>5</sub>) compared to RDF application (N<sub>2</sub>), respectively.

Significantly lowest grain yield (2335, 2274 and 2305 kg ha<sup>-1</sup> in 2015, 2016 and pooled mean, respectively) was recorded under absolute control (N<sub>1</sub>). Application of recommended dose of fertilizer NPK (N<sub>2</sub>) to the rice resulted in 102.95, 92.82 and 99.77% increase in grain yield during 2015, 2016 and pooled mean respectively over absolute control treatment (no fertilizer). RDF along with soil application of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (N<sub>3</sub>) recorded significantly higher grain yield (5187, 4993 and 5090 kg ha<sup>-1</sup> in 2015, 2016 and pooled mean, respectively) compared to only RDF application (4739, 4469 and 4604 kg ha<sup>-1</sup> in 2015, 2016 and pooled mean, respectively). Soil application of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (N<sub>3</sub>) increase grain yield in the tune of 9.44, 11.71 and 10.54% during 2015, 2016 and pooled mean respectively over NPK RDF application (N<sub>2</sub>). Two foliar application of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 0.5% (N<sub>4</sub>) was found better over soil application of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (N<sub>3</sub>) and resulted in 5.71, 3.29 and 4.52% increase in grain yield during 2015, 2016 and pooled mean, respectively over soil application of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup>.

Significant increase in grain yield due to nano zinc application is mainly attributed to significantly higher number of panicle m<sup>-2</sup>, panicle length, panicle weight, test weight, number of spikelets per panicle.

The increased yield attributes might be due to role of Zn in biosynthesis of Indole acetic acid (IAA) and especially due to its role in initiation of primordial reproductive parts and partitioning of photosynthates towards them. It is evident that application of Zn stimulated rice growth, enhanced the tiller production which was finally manifested in superior yield attributes. The increase in the number of panicles m<sup>-2</sup> might be attributed to adequate Zn supply which may have increased the supply of other nutrients and stimulated the overall plant growth. The increase in the number of grains panicle<sup>-1</sup> might have been owing to its enhancing effect on the physiological activities, photosynthesis and translocation and assimilation of photosynthates and formation of higher number of spikelets

during the spikelet initiation process which ultimately resulted in higher number of grains/panicle (Talib *et al.*, 2016) [14].

Significantly higher straw yield was recorded in RDF+ 2 FS of nano Zn @ 1000 ppm (N<sub>5</sub>) (7570, 7224 and 7397 kg ha<sup>-1</sup> during 2015, 2016 and pooled mean, respectively) compared to other nutrient management practices except RDF+ 2 FS of nano Zn @ 1500 ppm (N<sub>6</sub>). Significantly lowest straw yield (3270, 3320 and 3295 kg ha<sup>-1</sup> during both the year and pooled mean) was recorded under absolute control (N<sub>1</sub>) treatment.

### Zinc uptake

Direct seeded rice recorded significantly higher uptake of zinc in whole grain (203.13, 184.62 and 193.87 g ha<sup>-1</sup>) and straw (331.87, 309.94 and 320.91 g ha<sup>-1</sup>) over normal transplanted rice during 2015, 2016 and in pooled mean, respectively (Table 2). Under transplanted rice, the zinc uptake in whole grain was 179.90, 163.68 and 171.79 g ha<sup>-1</sup> and in straw, it was 292.57, 271.87 and 282.22 g ha<sup>-1</sup> during 2015, 2016 and in pooled mean, respectively. The per cent increase in total zinc uptake under direct seeded rice was 13.2, 13.5 and 13.4 % over transplanted rice during both the years and pooled mean, respectively. This is due to higher zinc content in whole grain and straw and significantly higher dry matter production under direct seeded rice compared to transplanted rice.

RDF+ 2 FS of nano Zn @ 1000 ppm (N<sub>5</sub>) and RDF+ 2 FS of nano Zn @ 1500 ppm (N<sub>6</sub>) treatments being on par with each other recorded significantly higher zinc uptake in grain and straw over other nutrient management practices. This is due to significantly higher content of Zn in grain and straw and higher dry matter production in N<sub>5</sub> and N<sub>6</sub> treatments compared to all other nutrient management practices (Singh and Tripathi, 2007) [12].

Significantly higher grain, straw and total zinc uptake was registered in RDF+ 2 FS of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 0.5% (N<sub>4</sub>) compared to RDF+ SA of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (N<sub>3</sub>).

During both the years of study, the total (grain+ straw) zinc uptake were in order of RDF+ 2 FS of nano Zn @ 1500 ppm (N<sub>6</sub>) > RDF+ 2 FS of nano Zn @ 1000 ppm (N<sub>5</sub>) > RDF+ 2 FS of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 0.5% (N<sub>4</sub>) > RDF+ SA of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (N<sub>3</sub>) > only RDF application (N<sub>2</sub>) > absolute control (N<sub>1</sub>) treatment (Raddy *et al.*, 2017) [10].

RDF+ SA of ZnSO<sub>4</sub>.7H<sub>2</sub>O @ 25 kg ha<sup>-1</sup> (N<sub>3</sub>) recorded significantly higher grain, straw and total (grain+ straw) zinc uptake compared to only RDF application (N<sub>2</sub>) and absolute control (no fertilizer) treatment (N<sub>1</sub>) during both the years. Absolute control (N<sub>1</sub>) treatment recorded lowest grain, straw and total (grain+ straw) zinc uptake compared to all other nutrient management practices due to lowest zinc content and dry matter production under absolute control (N<sub>1</sub>).

**Table 1:** Yield of rice as influenced by crop establishment methods and nutrient management practices (*rabi*, 2015 and 2016)

Treatments	Grain yield (kg ha <sup>-1</sup> )			Straw yield (kg ha <sup>-1</sup> )			Biological yield (kg ha <sup>-1</sup> )		
	2015	2016	Pooled	2015	2016	Pooled	2015	2016	Pooled
<b>Crop establishment methods (M)</b>									
M <sub>1</sub> - Direct Seeded Rice (DSR)	5198	4886	5047	6675	6447	6561	11873	11333	11598
M <sub>2</sub> - Normal Transplanted Rice (NTP)	4733	4489	4611	6000	5813	5907	10732	10303	10517
SEm <sub>±</sub>	98	83	89	121	109	101	217	163	169
CD (p=0.05)	441	373	401	545	492	455	977	733	761
<b>Nutrient Management practices (N)</b>									
N <sub>1</sub> - Absolute control (No fertilizer)	2335	2274	2305	3270	3320	3295	5605	5595	5587
N <sub>2</sub> - Control (RDF)	4739	4469	4604	6154	5981	6068	10893	10450	10671
N <sub>3</sub> -RDF+ SA of ZnSO <sub>4</sub> .7H <sub>2</sub> O @ 25 kg ha <sup>-1</sup>	5187	4993	5090	6593	6462	6527	11779	11455	11617
N <sub>4</sub> -RDF+ 2 FS of ZnSO <sub>4</sub> .7H <sub>2</sub> O @ 0.5%	5483	5157	5320	6902	6656	6779	12385	11813	12098
N <sub>5</sub> -RDF+ 2 FS of nano ZnO @ 1000 ppm	6070	5659	5878	7570	7224	7397	13640	12884	13261

N <sub>6</sub> -RDF+ 2 FS of nano ZnO @ 1500 ppm	5978	5574	5776	7537	7137	7337	13515	12710	13112
SEm <sub>±</sub>	134	109	111	147	114	121	259	149	242
CD (p=0.05)	386	315	320	424	328	349	747	430	699
<b>Interactions (MxN)</b>									
<b>Nutrient management at same level of crop establishment</b>									
SEm <sub>±</sub>	189	154	157	208	161	171	366	211	342
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Crop establishment at same or different level of nutrient management</b>									
SEm <sub>±</sub>	198	163	168	225	183	186	398	252	355
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
General Mean	4965	4688	4829	6337	6130	6234	11303	10818	11058
Coefficient of Variation (%)	9.7	8.7	9.0	9.4	8.7	7.9	9.4	7.4	7.5

RDF- Recommended Dose of Fertilizers (N, P<sub>2</sub>O<sub>5</sub>& K<sub>2</sub>O @ 120:60:40 kg ha<sup>-1</sup>); SA- Soil Application; FS- Foliar Spray; NS- Non Significant

**Table 2:** Zinc uptake in whole grain and straw of rice as influenced by crop establishment methods and nutrient management practices during *rabi*, 2015 and 2016 under field experiment

Practices	Zinc uptake in whole grain (g ha <sup>-1</sup> )			Zinc uptake in straw (g ha <sup>-1</sup> )			Total zinc uptake (g ha <sup>-1</sup> )		
	2015	2016	Pooled	2015	2016	Pooled	2015	2016	Pooled
<b>Crop establishment methods (M)</b>									
M <sub>1</sub> - Direct Seeded Rice (DSR)	203.13	184.62	193.87	331.87	309.94	320.91	535.01	494.56	514.78
M <sub>2</sub> - Normal Transplanted Rice (NTP)	179.90	163.68	171.79	292.57	271.87	282.22	472.47	435.55	454.01
SEm <sub>±</sub>	3.78	3.38	2.91	6.31	4.77	4.98	8.72	7.41	7.48
CD (p=0.05)	17.00	15.22	13.11	28.42	21.48	22.41	39.22	33.35	33.68
<b>Nutrient management practices (N)</b>									
N <sub>1</sub> - Absolute control (No fertilizer)	72.75	65.89	69.29	126.77	121.02	123.90	199.52	186.93	193.19
N <sub>2</sub> - Control (RDF)	166.41	149.30	157.86	278.40	259.82	269.11	444.81	409.12	426.97
N <sub>3</sub> -RDF+ SA of ZnSO <sub>4</sub> .7H <sub>2</sub> O @ 25 kg ha <sup>-1</sup>	195.30	180.35	187.83	316.88	299.13	308.00	512.18	479.48	495.83
N <sub>4</sub> -RDF+ 2 FS of ZnSO <sub>4</sub> .7H <sub>2</sub> O @ 0.5%	212.17	195.04	203.60	340.43	316.85	328.64	552.60	511.88	532.25
N <sub>5</sub> -RDF+ 2 FS of nano ZnO @ 1000 ppm	254.75	230.10	242.43	397.20	366.60	381.90	651.95	596.69	624.32
N <sub>6</sub> -RDF+ 2 FS of nano ZnO @ 1500 ppm	247.72	224.22	235.97	413.64	382.03	397.84	661.37	606.25	633.81
SEm <sub>±</sub>	4.81	4.46	3.57	7.00	5.84	5.96	11.54	8.83	7.91
CD (p=0.05)	13.89	12.88	10.30	20.21	16.88	17.22	33.33	25.51	22.85
<b>Interactions (MxN)</b>									
<b>Nutrient management at same level of crop establishment</b>									
SEm <sub>±</sub>	6.80	6.31	5.04	9.90	8.26	8.43	16.32	12.49	11.19
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Crop establishment at same or different level of nutrient management</b>									
SEm <sub>±</sub>	7.27	6.68	5.45	11.02	8.93	9.17	17.26	13.60	12.66
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
General Mean	191.52	174.15	182.83	312.22	290.91	301.57	503.74	465.06	484.40
Coefficient of Variation (%)	9.7	9.5	7.8	9.9	8.0	8.1	8.5	7.8	7.6

RDF- Recommended Dose of Fertilizers (N, P<sub>2</sub>O<sub>5</sub>& K<sub>2</sub>O @ 120:60:40 kg ha<sup>-1</sup>); SA- Soil Application; FA- Foliar Application; NS- Non Significant

## Conclusion

The study revealed that direct seeding through hand dibbling under puddled condition could be an option for rice cultivation as normal transplanting required more labour and delayed maturity compared to direct seeding. Application of RDF+ nano ZnO foliar application @1000 ppm is new innovation to increase zinc content and uptake and in enhancing grain yield over ZnSO<sub>4</sub>.7H<sub>2</sub>O application.

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