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## Agronomic fortification of rice and wheat grains with zinc for nutritional security

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**Zinc (Zn) deficiency is the most widespread micronutrient deficiency in crop plants and humans. Low intake of Zn through diet appears to be the major reason for the widespread prevalence of Zn deficiencies in human populations. Application of Zn fertilizer in soil having low Zn increased the grain yield in wheat up to 6.4–50.1%. However, soil Zn application increased the grain yield of rice only up to 7.2–14.8%.**

**Soil having sufficient Zn had no or little effect on grain yield with soil Zn application. The application of foliar Zn with or without propiconazole resulted in significant increases in grain Zn irrespective of soil Zn status. Application of foliar Zn along with propiconazole at earing and milk stages proved beneficial in increasing grain Zn content in both rice and wheat. Hence agronomic biofortification is possible and could be considerably economical if used along with a fungicide depending upon appearance of a disease.**

**Keywords:** Agronomic fortification, rice, wheat, zinc deficiency.

THE foodgrain production of the Indian subcontinent improved tremendously after the introduction of high-yielding, dwarf, fertilizer-responsive varieties of rice and wheat during 1966–68, progress in manufacture and consumption of chemical fertilizers, increase in irrigation facilities and development of rural infrastructure<sup>1</sup>. Rice and wheat constitute nearly two-thirds of the energy needs of humans in India<sup>2</sup>. Rice–wheat cropping system occupies about 10 million ha in the Indo-Gangetic Plains of India and is spread over the states of Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal<sup>1</sup>. This cropping system has led to a significant decline in the area under legumes, which are a rich source of proteins and micronutrients. Till recently, the three major micronutrient deficiencies recognized globally were vitamin A deficiency leading to blindness, iron deficiency causing anaemia, and iodine deficiency responsible for goitre. Deficiency of zinc (Zn) has received global attention recently. Graham and Welch<sup>3</sup> reported that 50% of the soil used for cereal production in the world contains a low level of plant available Zn, which reduces not only grain yield but also nutritional quality. Zn deficiency in humans causes a wide range of health complications, including impairment in the immune system, learning ability and physical growth, and increase in mortality and infections<sup>4</sup>. Children are particularly more sensitive to Zn deficiency, which has been shown to be a major cause of death among children all over the world. It is responsible for nearly 450,000 deaths in children under the age of 5 years, which corresponds to 4.4% of the deaths among children less than 5 years of age globally<sup>5</sup>.

Zn has been found useful in improving yield and yield components of wheat<sup>6–8</sup> and rice<sup>9</sup>. It is high time that along with food security due attention should also be given to adequate micronutrient nutrition. Soil and foliar Zn fertilization has shown good response in a number of crops including rice and wheat. Development of a new variety by conventional plant breeding is genetic biofortification, whereas agronomic biofortification is enrichment of micronutrients in the grains by application of appropriate fertilizers. Fertilization approach is cheaper, faster and safer and can be applied to a number of crops. The

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application of micronutrients at appropriate stage with the right concentration is important, which plays a major role in obtaining promising results. The present study was therefore conducted under field conditions in different ecological zones of Punjab, India, to investigate the role of soil and foliar applied Zn fertilizers in combination with commonly used fungicide, propiconazole 25 EC, in improving cereal grain Zn.

Rice genotype PR 120 was grown at all the locations, except Pusa 1121 (basmati rice) used in Bhagatpur in 2009. This short-statured and high-yielding variety has been developed for specific environments and cropping systems; it is of short duration and consumes less water. The wheat varieties used were PBW 550 and DBW 17, developed by Punjab Agricultural University, Ludhiana and Directorate of Wheat Research, Karnal respectively. These varieties are also short-statured and have higher harvest index.

Field experiments were conducted during June 2009 to September 2011 in different ecologies of Punjab. The soils were normal in pH and EC with low available N, medium in P and sufficient in K. The soils at Gurdaspur and Bathinda were low in diethylene triamine pentaacetic acid (DTPA) extractable Zn ( $<0.6 \text{ mg kg}^{-1}$ ), but not at other sites.

The region has a subtropical climate, with hot, wet summers for rice and cool, dry winters for wheat. Average annual rainfall is 734 mm, constituting 44% of pan evaporation. The depth to the groundwater is over 15 m.

The experiment was initiated with rice crop in 2009 in Punjab representing the Trans Indo-Gangetic Plains (IGP) of India. It comprised of two treatments, i.e. no Zn and soil Zn application ( $50 \text{ kg ZnSO}_4 \cdot 7\text{H}_2\text{O ha}^{-1}$ ) in a randomized complete block design with four replicates. From wheat season (2009–10), additional treatments were added to the experiment, i.e. (i) no Zn, (ii) application of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  in the soil ( $50 \text{ kg ha}^{-1}$ ) along with foliar Zn application at 0.5% of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (at earing and at milk stage), and (iii) treatment (ii) + application of Zn along with propiconazole fungicide (F). In 2010–11, one more treatment ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O ha}^{-1}$  @  $50 \text{ kg ha}^{-1}$  in the soil) was added for both wheat and rice.

Plot size was  $10 \text{ m} \times 2 \text{ m}$ , with earth bunds around each plot to avoid movement of Zn fertilizer across the plots. After pre-irrigation to both rice and wheat, the field was ploughed with tractor-driven harrows and cultivators. Rice was seeded ( $20 \text{ kg ha}^{-1}$ ) on the beds for raising nursery. The nursery of 30 days age was transplanted manually in the experiment. Irrigation water was kept standing in rice for the first two weeks after transplanting and later irrigation was applied two days after evaporation of water in the field. Rice was transplanted during all the years in June, keeping row-to-row spacing of 20 cm and plant-to-plant of 15 cm. Zn fertilizer was applied before transplanting of rice crop. No P and K fertilizers were applied

to rice crop, but N was applied @  $120\text{--}150 \text{ kg ha}^{-1}$  at different locations. Where the soil was loamy sand (Ferozepur and Ludhiana), higher fertilizer dose ( $150 \text{ kg ha}^{-1}$ ) was used. To rice crop, one-third of N was applied at sowing, the remaining N was top-dressed in two equal split doses, i.e. 3 and 6 weeks after transplanting of the nursery. Weeds were well controlled in rice using Butachlor 50 EC within two days of transplanting of the crop as pre-emergence application. Rice crop was harvested during 15–22 October.

Wheat (cultivar PBW 550 2009–10/DBW 17 2010) was sown using  $112.5/100 \text{ kg ha}^{-1}$  seed rate with seed-cum-fertilizer drill at 20 cm of row spacing. Fertilizer dose of  $150 \text{ kg ha}^{-1}$  N was applied to wheat at Ludhiana and Bathinda, while  $120 \text{ kg ha}^{-1}$  of N was applied at other locations. Phosphorus @  $26 \text{ kg P ha}^{-1}$  was applied at the time of sowing. To wheat, half of N and full dose of P were applied at sowing and the remaining N was top-dressed with first irrigation. Urea and diammonium phosphate (DAP) were used as the source of nutrients. In wheat, Arelon 75WP (isoproturon) at  $1.25 \text{ kg ha}^{-1}$  (35 DAS) followed by 2,4-D sodium salt (80%) at  $0.625 \text{ kg ha}^{-1}$  were used for controlling both grass and broadleaf weeds respectively. Both rice and wheat were harvested manually and the stubbles were removed from ground level. Wheat crop was harvested between 6 and 14 April during different years of the study period.

Each plot was harvested leaving non-experimental area for grain and straw yields. The rice crop was harvested and threshed manually, but wheat was harvested manually and threshed with machine.

The threshing was done with hands for determination of Zn in the grains. Grain was analysed with an atomic absorption spectrophotometer (model AAS FS 240 Varian, Australia) using standard procedures. Soil Zn and grain Zn were analysed in different laboratories to ensure precision.

The economic analysis was carried out using actual expenditure of activities/input and the prevailing rates for rice and wheat crops produced separately. The gross and net returns were calculated as follows.

Gross returns = Crop yield (t/ha) price of the produce (US\$).

Net returns = Gross returns (US\$) – cost of cultivation (US\$).

The net returns were presented graphically across the locations.

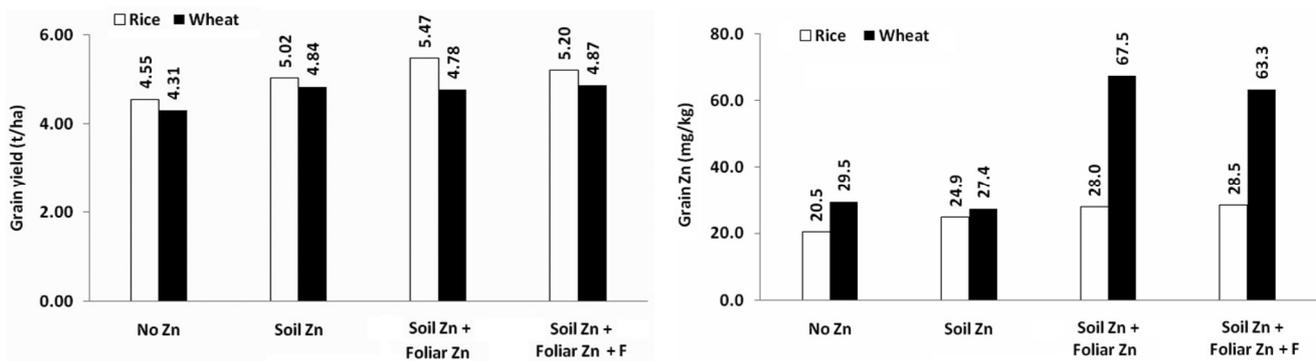
The data collected were analysed using analysis of variance (ANOVA) by IRRISTAT v. 5.0 and Genstat v. 7.1. Treatment means was compared by DAP least significant difference (LSD) at  $P = 0.05$ .

In 2009, rice grain yield obtained at Bhagatpur was less compared to other locations; this was due to basmati

**Table 1.** Effect of various Zn fertilizer treatments on grain yield ( $t\ ha^{-1}$ ) of rice during 2009–11 at three locations in Punjab, India

Location	2009			2010				2011				
	No Zn	Soil Zn	LSD ( $P = 0.05$ )	No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( $P = 0.05$ )	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( $P = 0.05$ )
Patiala	5.12	5.47	0.25	5.22	5.82	5.92	NS	5.21	5.71	5.81	5.92	0.21
Ferozpur	4.08	4.30	0.10	4.32	4.93	4.89	NS	4.53	4.89	4.92	5.03	0.22
Bhagatpur	2.87	3.17	0.14	4.53	5.24	5.33	NS	5.02	5.66	5.83	5.91	0.41
Mean	4.02	4.31	0.17	4.72	5.33	5.42	NS	4.92	5.42	5.52	5.20	0.31
% Increase		7.21			12.92	14.83			10.16	12.20	5.69	

NS, Not significant.

**Figure 1.** Effect of various zinc fertilizer treatments on the grain yields and grain zinc of rice and wheat across locations and years.

variety (Pusa 1121) sown at the location (Table 1). Application of soil Zn significantly improved grain yield at all the locations. The highest response in grain yield over no Zn was recorded at Bhagatpur (10.5%), followed by Patiala (6.8%) and Ferozpur (5.4%). In 2010, the results were non-significant but the trend was in favour of soil Zn application. In 2011, the highest grain yield was recorded in soil Zn + foliar Zn + propiconazole, which was statistically at par with soil Zn and soil + foliar Zn. On the basis of pooled mean grain yield over the locations, the increase was 7.2% in 2009, 12.9% in 2010 and 10.2% in 2011. On the basis of mean across the years and locations, soil Zn application, soil Zn + foliar Zn and soil Zn + foliar Zn + propiconazole increased the grain yield by 10.3%, 20.2% and 14.3% respectively. This may be due to response of rice crop to soil Zn application in marginal soils (Figure 1).

In 2009, the increase in rice grain Zn with soil Zn application over no Zn was in the order Ferozpur > Patiala > Bhagatpur (Table 2). Application of soil Zn resulted in lesser increase in the grain Zn compared to foliar Zn application. This may be due to less uptake of Zn from the soil by the rice roots. Soil application could not develop the Zn gradient in leaves to translocate Zn to the developing rice grains. In 2010, at Ferozpur and Bhagatpur the grain Zn content in soil Zn + foliar Zn +

propiconazole was statistically on par with soil Zn + foliar Zn, but significantly higher than no Zn application. This may be due to non-appearance of diseases at these locations. During 2011, at Patiala and Ferozpur the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole but significantly higher than no Zn and soil Zn treatments. Soil Zn also slightly increased the grain Zn, but it was not significantly different from no Zn treatment. However, at Bhagatpur the grain Zn was highest for soil Zn + foliar Zn + propiconazole treatment. This indicates the compatibility of foliar Zn + propiconazole in rice crop, which also saves the separate cost involved in the application of foliar Zn for grain Zn enrichment. On the basis of mean across all the locations both soil Zn + foliar Zn and soil + foliar Zn + propiconazole treatments recorded similar grain Zn, which was significantly higher than soil Zn and no Zn treatments. Foliar Zn applications near flowering stage may be one way to increase Zn in grains as the uppermost leaves were readily exposed to foliar sprays<sup>9</sup>. The spraying of 3.0 kg  $ZnSO_4 \cdot 7H_2O$  in two applications at the flowering and early grain development stages increased the grain Zn significantly irrespective of the soil types. The results suggest that the negative effect of low DTPA extractable soil Zn status on grain Zn concentrations is not overcome

## RESEARCH COMMUNICATIONS

**Table 2.** Effect of various Zn fertilizer treatments on grain zinc (mg kg<sup>-1</sup>) of rice during 2009–11 at three locations in Punjab, India

Location	2009			2010				2011				
	No Zn	Soil Zn	LSD ( <i>P</i> = 0.05)	No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( <i>P</i> = 0.05)	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( <i>P</i> = 0.05)
Patiala	24.1	28.5	3.62	18.1	26.5	26.4	4.2	20.1	22.1	28.6	28.5	3.3
Ferozepur	18.3	22.1	2.90	22.2	29.5	29.6	5.1	21.2	23.1	27.9	27.8	3.9
Bhagatpur	21.5	24.4	2.39	18.2	25.9	26.1	5.3	20.6	21.9	29.1	29.2	3.7
Mean	21.3	25.0	3.0	19.5	27.3	27.4	4.9	20.6	22.4	28.5	28.5	3.6
% Increase		17.4			40.0	40.5			8.7	38.3	38.4	

**Table 3.** Effect of various Zn fertilizer treatments on grain yield (t ha<sup>-1</sup>) of wheat during 2009–11 at five locations in Punjab, India

Location	2009–10					2010–11				
	No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( <i>P</i> = 0.05)	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( <i>P</i> = 0.05)	
Zn-deficient	Bathinda	4.40	4.78	4.89	0.31	2.18	4.79	4.75	4.81	0.91
	Gurdaspur	4.54	4.73	4.95	0.15	4.23	4.83	4.83	5.03	0.16
	Mean	4.47	4.76	4.92	0.23	3.21	4.81	4.79	4.92	0.54
	% Increase		6.41	10.03			50.08	49.45	53.51	
Zn-sufficient	Ferozepur	4.13	4.23	4.33	NS	4.33	4.45	4.46	4.48	NS
	Patiala	4.80	5.10	4.91	NS	5.02	5.2	5.23	5.35	NS
	Bhagatpur	4.61	4.75	4.69	NS	4.86	4.95	4.93	5.23	0.13
	Mean	4.51	4.69	4.65	NS	4.74	4.87	4.87	5.02	0.04
	% Increase		3.97	2.92			2.74	2.89	5.98	
Overall mean		4.50	4.72	4.75	0.20	4.12	4.84	4.84	4.98	0.16
% Overall increase			4.94	5.75			17.46	17.36	20.76	

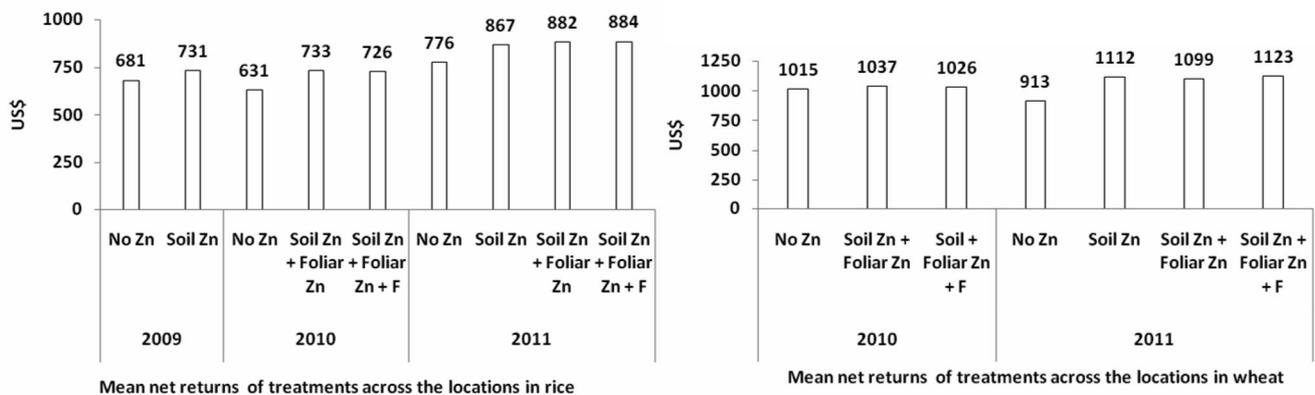
by application of Zn fertilizer at doses recommended for better yields of rice and wheat. Similar results were also reported elsewhere<sup>10</sup>. The best way of biofortification is through supplying the biofortified rice and wheat to reach rural populations in remote areas of India, such as IGP, who may not consume biofortified processed foods<sup>11</sup>. Farmers apply Zn fertilizer only if they expect a yield response as obtained in the present study. The foliar application of Zn in cereals at flowering or early grain development stages is uncommon in India, as there is no yield response. So when the farmers apply fungicides like propiconazole for control of various diseases in rice, zinc sulphate heptahydrate can be mixed with the fungicide to get positive effect of fortification of Zn in cereal grains. Based on the overall mean across years and locations, soil foliar Zn application without or with fungicides increased the grain Zn content by 21.5–39.0% over no Zn application (Figure 1).

The response of wheat grain yield to soil Zn application was observed in the order Bathinda > Patiala > Gurdaspur > Bhagatpur > Ferozepur (Table 3). At Bathinda grain yield recorded in soil Zn + foliar Zn + propiconazole was statistically on par with soil Zn + foliar Zn, but significantly higher than no Zn application. However, at Gurdaspur the highest grain yield was recorded in soil Zn + foliar Zn + propiconazole, which was significantly

higher than soil Zn + foliar Zn and no Zn. The grain yield recorded in soil Zn + foliar Zn was significantly higher than no Zn. Although wheat grain yields at Ferozepur, Patiala and Bhagatpur were similar, the trend was similar as recorded at Bathinda and Gurdaspur. During 2010–11, the grain yield recorded at Patiala and Ferozepur was non-significant. This is because yellow rust could not infect the experimental crop during that year of experimentation. At Bathinda, high soil pH and high levels of CaCO<sub>3</sub> and low levels of organic matter and soil moisture were predominantly responsible for low availability of Zn to plant roots. It has also been reported<sup>6</sup> that Zn application improved biological yield as well as grain yield of wheat grown on calcareous soils as shown in the present study as well. Higher grain yield of wheat was reported with the application of Zn in the soils<sup>12,13</sup>. At Gurdaspur the grain yield was highest in soil Zn + foliar Zn + propiconazole, which was significantly higher than the rest of the treatments. This may be because the fungicide was able to control the yellow rust disease in wheat. At Bhagatpur the grain yield recorded in soil Zn + foliar Zn + propiconazole was significantly higher than no Zn, soil Zn and soil Zn + foliar Zn. On the basis of pooled mean across soil-deficient Zn locations, the highest grain yield was recorded in soil Zn + foliar Zn + propiconazole, which was 10.0% higher in 2009–10 and 53.5% higher

**Table 4.** Effect of various Zn fertilizer treatments on grain zinc ( $\text{mg kg}^{-1}$ ) of wheat during 2009–11 at five locations in Punjab, India

Location		2009–10				2010–11				
		No Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( $P = 0.05$ )	No Zn	Soil Zn	Soil Zn + foliar Zn	Soil Zn + foliar Zn + propiconazole	LSD ( $P = 0.05$ )
Zn-deficient	Bathinda	25.0	81.0	70.0	13.5	22.7	24.7	72.2	73.5	18.4
	Gurdaspur	34.3	69.8	61.3	4.8	25.3	27.2	60.4	60.3	13.9
	Mean	29.6	75.4	65.6	9.1	24.0	25.9	66.3	66.9	16.1
	% Increase		154.4	121.5			7.9	175.8	178.5	
Zn-sufficient	Ferozepur	48.7	63.0	65.3	NS	26.5	28.7	61.6	61.9	18.2
	Patiala	34.0	64.0	53.7	17.2	30.2	31.2	61.2	59.2	16.6
	Bhagatpur	25.8	77.0	64.8	12.2	22.4	25.3	64.2	63.2	13.0
	Mean	36.1	68.0	61.3	NS	26.3	28.4	62.4	61.4	15.9
	% Increase		88.2	69.5			7.9	136.7	133.2	
Overall mean		33.5	71.0	63.0	0.2	25.4	27.4	63.9	63.6	0.2
% Overall increase			111.6	87.9			7.9	151.5	150.3	

**Figure 2.** Effect of various zinc fertilizer treatments on net returns (US\$) of rice and wheat.

than no Zn application. It was statistically similar to grain yield obtained in soil Zn + foliar Zn treatment. The highest grain yield on the pooled mean across the locations recorded in soil Zn + foliar Zn + propiconazole may be due to proper control of yellow rust disease. The positive effect of Zn applications on plant growth leading to increased leaf area index, plant height, number of fertile tillers  $\text{m}^{-2}$ , number of filled spikelets/spike, spike length, grains/spike, biological and straw yields and 1000-grain weight culminating in improved grain yield were also reported<sup>14</sup>. The percentage increase in grain yield in Zn-deficient soils was more than Zn-sufficient soils. This may be due to the effect of Zn fertilizer to make more available Zn to the crops, which ultimately increased the grain yield. Wheat grain yield across the years and locations increased from 10.9% to 13.0% in soil Zn + foliar Zn with or without fungicide (Figure 1).

During 2009–10, wheat grain Zn was significantly higher at all the locations except at Ferozepur (Table 4). The highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole, but significantly higher than no Zn appli-

cation at all the locations, except Ferozepur, where the highest grain Zn was significantly higher than soil Zn + foliar Zn + propiconazole. On the basis of mean at Zn-sufficient locations, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole but significantly higher than no Zn application. During 2010–11, again the highest grain Zn was recorded in soil Zn + foliar Zn, except at Bathinda and Ferozepur, where the highest grain Zn was recorded in soil Zn + foliar Zn + propiconazole. At Bhagatpur the grain Zn was similar in both soil Zn + foliar Zn and soil Zn + foliar Zn + propiconazole treatments. At Gurdaspur and Patiala, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn, but significantly higher than no Zn treatment. On the basis of overall mean across the locations, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole. Under Zn-deficient conditions (Bathinda), Zn concentration in the grains was lower compared to the other locations having sufficient soil Zn. On the basis of overall mean the percentage

increase in grain Zn in wheat with soil Zn + foliar Zn application alone or in combination with propiconazole was higher (121.5%–154.4% in 2009–10 and 175.8%–178.5% in 2010–11) in Zn-deficient soils compared to Zn-sufficient soils (69.5%–88.2% in 2009–10 and 133.2%–136.7% in 2010–11). The grain Zn in the Zn-deficient soil at Bathinda and Gurdaspur was low due to less available DTPA-extractable Zn. On the basis of overall mean, the highest grain Zn was recorded in soil Zn + foliar Zn, which was statistically on par with soil Zn + foliar Zn + propiconazole in 2010–11. This may be due to non-appearance of yellow rust at most of the locations. The grain Zn concentration was not influenced by fertilizer Zn application in the soil. This may be because translocation from soil to plant leaves is not so significant even at low DTPA-extractable sites. On the basis of mean across the locations and years, the foliar application of Zn with or without fungicide increased the grain Zn from 114.6% to 128.8% over no Zn application (Figure 1). Propiconazole is effective against yellow rust in wheat<sup>15</sup>. Higher order increase of grain Zn in deficient soil Zn locations may be due to enhanced effect of soil as well as foliar Zn applications<sup>16,17</sup>.

In rice, during 2009, soil Zn application recorded the higher returns (Figure 2). During 2010, soil Zn + foliar Zn recorded the highest or similar returns as recorded in soil Zn + foliar Zn + propiconazole, but was higher than no Zn application. During 2011, soil Zn + foliar Zn + propiconazole recorded the highest returns across all locations. The higher returns in soil Zn or soil Zn + foliar Zn and soil Zn + foliar Zn + propiconazole may be due to higher grain yields recorded with the treatments.

Across the locations, during 2010, the net returns in wheat were highest with soil Zn + foliar Zn. Soil Zn + foliar Zn + propiconazole recorded the highest net returns in 2011. This was due to higher grain yield of wheat recorded in this treatment.

Thus soil Zn application increased wheat grain yield up to 50% and rice grain yield only up to 14.8%. Soils rich in Zn showed no or little effect on grain yield when Zn was applied. Application of foliar Zn with or without propiconazole resulted in significant increase in grain Zn, which was 38–40% in rice and 87–150% in wheat, over no Zn. Foliar Zn along with propiconazole fungicide at earing and milk stages proved most beneficial in increasing Zn content in the grains. This not only helped to increase grain Zn but was also most economical. However, fungicide application will depend upon the appearance of a disease and hence has value under specific conditions.

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