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An effective strategy to improve grain zinc concentration of winter wheat, Aphids prevention and farmers' income

Xiao-Zhong Wang[a], Dun-Yi Liu[a], Wei Zhang[a], Cheng-Ju Wang[b], Ismail Cakmak[c], Chun-Qin Zou[a,*]

[a] *Key Laboratory of Plant–Soil Interactions, Ministry of Education, Center for Resources, Environment and Food Security, China Agricultural University, Beijing 100193, PR China*

[b] *Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100193, PR China*

[c] *Faculty of Engineering and Natural Sciences Sabanci University, 34956 Istanbul, Turkey*

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ABSTRACT

Foliar zinc (Zn) application represents an effective way to improve grain Zn concentration of cereal crops. Spraying Zn together insecticides would be an important advantage for the growers to minimize costs. However, there is very little information about combined foliar application of Zn fertilizers together with insecticides. A field experiment was conducted to investigate how spraying Zn fertilizer in form of $ZnSO_4$ together with commonly applied insecticides (e.g., Acetamiprid and Imidacloprid) affects the grain Zn concentration of wheat grown in the Northern China Plain. The main treatment included three levels: only insecticide applied (recorded as T1); foliar Zn fertilizer applied one day after insecticide application (recorded as T2); foliar Zn fertilizer applied together with insecticide (recorded as T3). The results showed that there was no antagonistic effect between insecticide and Zn when they were sprayed together. Compared with insecticide application only, foliar application of Zn fertilizer with insecticide increased grain yield by 3.7–4.9%, and grain Zn density by 48.2–61.6%. Grain yield and grain Zn concentration were not affected no matter Zn fertilizers were applied simultaneously with insecticide or one day after. The toxic effect of insecticide on insects was not affected when it was mixed with Zn fertilizer in the laboratory condition. Compared with application of Zn fertilizer and insecticide at different times, simultaneous combination can reduce the cost by 120 USD ha^{-1} and increase farmers' net income by 6.3%. Therefore the results show clearly that $ZnSO_4$ can be mixed and sprayed together with insecticide without causing any adverse effect. This is a very useful and cost-effective approach for growers contributing both to improving grain yield and grain Zn concentration and controlling aphids as well.

1. Introduction

Zinc (Zn) deficiency in crops and humans is a serious problem affecting both crop production and human health (Welch and Graham, 2004; Cakmak, 2008). Globally, 50% of cereal-growing soils are Zn-deficient and about 40% of agricultural soils are Zn-deficient in China (Yang et al., 2007). It is estimated that Zn deficiency also affects more than 1/3 of people worldwide (Stein, 2010), and ranks fifth of the leading causes of disease in low-income countries according to the World Health Organization (WHO, 2002).

Low dietary intake of Zn has greatly contributed to the widespread occurrence of Zn deficiency in humans (Cakmak et al., 2010b; Zhang et al., 2012). Cereal crops play an important role in satisfying daily calorie intake in developing world. However, Zn contents of cereal-based foods are inherently inadequate to meet human demands, particularly when grown on Zn-deficient soils (Cakmak, 2008; Boonchuay et al., 2013). As one of the three globally major cereal crops, wheat represents a main dietary source of calories, proteins and Zn for the humans. Wheat is responsible for up to 70% of daily calorie intake in the rural parts of developing countries (Cakmak, 2008; Shewry, 2009). China is the biggest producer of wheat in the world and the North China Plain (NCP) contributes about 70% of the national wheat production (Liu et al., 2010). Wheat-based food products supply more than 20% of the daily Zn intake in China, especially in rural areas (Ma et al., 2008). Therefore, improving the Zn concentration of wheat is a very important challenge for the health of human populations living in the rural areas in China.

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[*] Corresponding author. Fax: +86 10 62731016.
E-mail address: zcq0206@cau.edu.cn (C.-Q. Zou).

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Breeding new cereal genotypes with high Zn (e.g., genetic biofortification) and applying Zn fertilizers (e.g., agronomic biofortification) are two important agricultural approaches used for improving human nutrition with Zn (Pfeiffer and McClafferty, 2007; Cakmak et al., 2010a). In case of genotypes with high genetic capacity for root absorption, shoot transportation and seed deposition of Zn, breeding and Zn fertilizers strategies represent synergistic and complimentary solution to the problem (Cakmak, 2008). Agronomic biofortification represents a short-term, globally applicable, and effective strategy in minimizing Zn deficiency-related health problems (Cakmak, 2008). It has been well-documented that foliar Zn application is much more effective than soil Zn application in improving grain Zn concentration of wheat which can increase significantly, especially in Zn-deficient soils (Yilmaz et al., 1997; Cakmak et al., 2010b; Zhang et al., 2012 Zou et al., 2012). Zinc sulfate ($ZnSO_4$) is recommended as the most effective Zn fertilizer for foliar application to increase grain Zn concentration and content (Cakmak, 2008; Wei et al., 2012).

Wheat aphids (e.g., *Macrosip humavenae*) are one of the most destructive and common pests which annually emerge in the wheat planting area in China (Luo et al., 2013). Controlling wheat aphids are vital because aphids are a major biotic constraint to wheat yield by the direct effect of their feeding (Crespo-Herrera et al., 2013). Foliar insecticides are a common, simple, and effective means of controlling the wheat aphids (Pike et al., 1993). The insecticides (e.g., Acetamiprid and Imidacloprid) are most commonly used insecticides controlling wheat aphids in many countries including China (Nakayama et al., 1997; Flückiger et al.,1992; Ahmed et al., 2001). The spraying time of Zn application is very important to achieve higher grain Zn concentration of wheat. The late growth stages (e.g., booting, anthesis and milk stages) are regarded as the best application times to maximize the grain Zn accumulation (Cakmak et al., 2010b). And according to our field survey, wheat aphids begin to occur at the seeding stage, and farmers generally spray the insecticide at the boot stage, anthesis stage and milk stage because those are the stages with the peak infestation. So the timing of foliar Zn application and foliar insecticide is almost synchronous. Among the 111 publications from peer review journals during 1990–2014 related to Zn foliar application of wheat, most of them adopted a separate foliar application of the Zn fertilizer and an insecticide. This practice doubles the labor costs, compared to a combined application approach. Little is known about the effectiveness of applying an insecticide and a Zn foliar application simultaneously. Therefore, the objective of this study was to test whether a foliar Zn fertilizer could be applied together with an insecticide to improve the grain Zn concentration of winter wheat without reducing the effect of the insecticide, and further to improve the farmers' net income.

2. Material and methods

2.1. Field locations and materials

Field experiments were conducted at two locations in Quzhou of Hebei province in the Northern China Plain (recorded as Location 1 and Location 2) during 2012–2013 winter wheat cropping season. The location 1 is situated at the experimental station of China Agricultural University (36.9°N,115.0°E), and the Location 2 is at Li Zhuang village (36.9°N,115.0°E), which is farmer's field. The basic information of the soils at both sites is presented in Table 1. The wheat cultivar used was Liangxing99 (*Triticum aestivum* L.) which is the most commonly cultivated variety in the area.

2.2. Field trial treatments

The same field plot experiment treatments were applied at both locations. The main treatment included three levels: only insecticide applied (recorded as T1, which is the normal approach of insecticide applied by farmers in wheat production); foliar Zn fertilizer applied one day after insecticide application (recorded as T2, which is the approach that the insecticide and fertilizer are applied separately and it was used method in the study of Zn biofortification in previous reports); foliar Zn fertilizer applied together with insecticide (recorded as T3, which represents the combination of insecticide and fertilizer and this is the approach to be put forwarded in our study). Subplot treatments included two types of insecticides: Acetamiprid [5% active ingredient content, Emulsifiable Concentrate (EC) type] and Imidacloprid [10% active ingredient content, Wettable Powder (WP) type] which are commonly used for wheat aphids control in China. The layout of the experiments was plots in randomized block design with four replicates. Thus, there were 24 plots with 35 m^2 (3.5 m × 10 m at Location 1; 7 m × 5 m at Location 2) at each location. Foliar Zn fertilizer was applied at a rate of 0.4% (w/v) $ZnSO_4{\bullet}7H_2O$ (23% Zn). According to the recommended application rate, the concentration of foliar applied Acetamiprid and Imidacloprid was 0.15% (v/v) and 0.075 % (v/v), respectively. 0.01% (v/v) Tween 20 was added in all foliar solutions and all treatments were sprayed by hand spray with small machine at the booting stage, anthesis and early milking stage, respectively. The solution volume of 800 L ha^{-1} was used in all treatments. The spray was conducted after sunset in days without wind (<0.2 m s^{-1}).

At Location 1, starting fertilizers included 100 kg N ha^{-1} as urea, 120 kg P_2O_5 ha^{-1} as calcium superphosphate, and 100 kg K_2O ha^{-1} as potassium sulfate and were surface applied. Before the jointing stage, 120 kg N ha^{-1} as urea was topdressed. At Location 2, starting fertilizers included 112.5 kg N ha^{-1}, 112.5 kg P_2O_5 ha^{-1} and 112.5 kg K_2O ha^{-1} as compound fertilizer (15–15–15) and were surface applied. Before the jointing stage, 70 kg N ha^{-1} as urea was topdressed. Field water condition was managed by flood irrigating three times at pre-wintering, jointing and flowering stage with 50 mm applied each time at each location.

2.3. Sample collection and analysis

The number of grains $spike^{-1}$ was determined by counting the grains on every spike from 30 randomly selected plants in each plot before harvest. At harvest, 6 m^2 (3 m × 2 m) wheat plants in the center of each plot were harvested to determine the grain yield and biomass. The grain samples were washed quickly by tap water and then deionized water, and then dried in an oven at 60–65 °C, and were ground with a steel stainless grinder (RT-02A) for mineral analysis. The Zn concentration was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after being digested with HNO_3–H_2O_2 by a microwave accelerated reaction system (CEM, Matthews, USA). IPE 684 grain (Wageningen University, The Netherlands) was used as reference materials.

Table 1
Basic information of tested soil.

Location	Soil texture	Total *N* (g kg^{-1})	Olsen *P* (mg kg^{-1})	NH_4OAc-exchangeable *K* (mg kg^{-1})	Organic matter (g kg^{-1})	pH	DTPA-Zn (mg kg^{-1})
Location 1	Clay loam	0.97	33.93	275	14.74	7.86	1.23
Location 2	clay loam	0.72	24.52	157	12.55	8.31	0.63

Table 2
Three-way analysis of variance (ANOVA) of the effects of location, insecticide and Zn and their interactions on tested parameters of winter wheat in the field.

Source of variation	Grain number (spike^{-1})	Thousand kernel weight (g)	Grain yield (Mg ha^{-1})	Yield harvest index (%)	Grain Zn concentration (mg kg^{-1})	Grain Zn content (g ha^{-1})
Locations (L)	ns[a]	ns	***	***	ns	ns
Insecticide types (I)	ns	ns	ns	ns	ns	ns
Insecticide and Zn foliar application methods (IZ)	ns	ns	***	ns	***	***
L*I	ns	ns	ns	ns	ns	ns
L*IZ	ns	ns	*	ns	ns	ns
I*IZ	ns	ns	ns	ns	ns	ns
L*I*IZ	ns	ns	ns	ns	ns	ns

[a] Levels of significance are represented by $^{*}P<0.05$ level. ** $P<0.01$ level. $^{***}P<0.001$ level and ns (not significant), $1>P>0.05$.

Table 3
Effects of locations and application of insecticide with or without Zn fertilizer on yield and yield components of winter wheat in the field.

Treatment	Grain number (spike^{-1})	Thousand kernel weight (g)	Grain yield (Mg ha^{-1})	Yield harvest index (%)
Locations				
Location 1	34 a[a]	35.5 a	8.8 a	41.3 b
Location 2	34 a	36.2 a	8.1 b	47.2 a
Insecticide and Zn foliar application methods				
T1	34 a	35.8 a	8.2 b	44.3 a
T2	33 a	35.9 a	8.6 a	44.4 a
T3	33 a	35.9 a	8.5 a	44.1 a

[a] Values followed by different letters in each column indicate significant differences at $P \leq 0.05$ by the LSD test. T1 represents the only insecticide applied treatment, T2 represents the foliar Zn fertilizer applied one day after insecticide application, T3 represents foliar Zn fertilizer applied together with insecticide.

2.4. Economic analysis

Costs (T1) = costs of (Plowing + Harrow + Seed + Sowing + Fertilizer + Irrigation + Herbicide + Harvest + Insecticide + spray labor);

Costs (T2)= Cost (T1) + costs of (Zn fertilizer + spray labor);

Costs (T3) = Cost (T1) + cost of Zn fertilizer;

Net income (USD ha^{-1}) = Wheat yield × Price of grain-Costs;

Income increase (T2 or T3, USD ha^{-1}) = Net income (T2 or T3) – Net income (T1);

Relative income increase (T2 or T3, %) = [Net income (T2 or T3) – Net income (T1) /Net income (T1) × 100.

The cost of Plowing, Harrow, Seed, Sowing, Fertilizer (N, P, and K fertilizer), Irrigation, Herbicide and Harvest is 110, 49, 37, 123, 442, 590, 32 and 123 USD ha^{-1}, respectively. The cost of insecticide, Zn fertilizer and spray labor (three times) is 37, 15, 120 USD ha^{-1}, respectively. Those data are the local farmer average survey data in 2012–2013 in the Hebei province. These calculations are based on Meng et al. (2012).

2.5. Laboratory toxicity test

In order to investigate whether toxic effect of insecticides on aphids is affected from addition of $ZnSO_4$, a laboratory test has been conducted by using different insecticide concentrations. The test measured insect body immersion method (Laecke et al., 1995). The tested insecticide concentrations were: 6.25, 12.5, 25, 50 and 100 mg L^{-1} for Imidacloprid and 4.9, 7.4, 11.1, 16.7, 25.0 mg L^{-1} for Acetamiprid. Each insecticide rate included two treatments: insecticide only and insecticide with Zn fertilizer (0.4% $ZnSO_4{\bullet}7H_2O$, Zn 23%). Each treatment was replicated four times. Each insecticide was diluted with 0.01% Tween20 in water solution to a prescribed concentration. The wheat leaf with 20 aphids each replication was dipped for 10 or 5 s. into each solution, and then the respective LC_{50} (median lethal concentration, mg L^{-1}) was calculated from the mortality at 6 h.

2.6. Data analysis

SAS software (8.0, USA) was used for statistical analysis. Data were analyzed with a three-factor and two-factor ANOVA procedure for plot design. Means were separated by Fisher's protected least significance difference (LSD) at $P<0.05$.SPSS software (17.0) was used for calculating LC_{50}.

3. Results

3.1. Grain yield, yield components and harvest index

Grain yield was significantly affected by Zn foliar application (Tables 2 and 3). Compared with the T1 treatment, grain yield of T2 and T3 treatments was increased by 4.9% and 3.7% at both locations, respectively. Compared with Location 2, grain yield of Location 1 was increased by 8.6%. There was no significant difference in grain yield between the T2 and T3 treatments at both locations (Table 3). Grain number per spike, thousand kernel and yield harvest index of winter wheat were not significantly affected by Zn foliar application together with insecticide or one day after insecticide application under given experimental conditions (Tables 2 and 3). The grain yield and harvest index were different between the two locations (Tables 2 and 3). Except grain yield, there was no interaction between location and foliar strategy of Zn and insecticide for grain number, thousand kernel or harvest index (Table 3). Insecticide types as well as their interactions with locations and Zn fertilizer did not significantly affect grain number, thousand kernel, grain yield or harvest index of winter wheat (Table 2).

3.2. Grain Zn concentration and content

Grain Zn concentration and content (e.g., total uptake by grains) were also significantly affected by foliar spray strategy of Zn and insecticide (Table 2, Fig. 1). At the Location 1,compared with T1, grain Zn concentration was increased by 59.2% and 82.9% for T2 and T3 treatments, respectively. There was a significant difference in grain Zn concentration between the T2 andT3 treatments. When compared with the T2 treatment, grain Zn concentration of the plants treated with T3 was increased by 14.9% (Fig. 1a). Different from Location1, there was no significant difference in grain Zn concentration between T2 and T3 treatments (Fig. 1a) and grain Zn concentration was increased by 39.3% and 44.1% for T2 and T3 treatments, respectively, compared with T1 treatment at Location 2. The results with the Zn content were similar to those results with Zn concentration (Fig. 1). At the Location 1, compared with T1 treatment, grain Zn content was increased by 63.5% and 88.5% for T2 and T3 treatments, respectively. There was also a significant difference in grain Zn content between the T2 and T3 treatments.

Table 4
Toxicity evaluation of two insecticides with and without Zn from laboratory test.

Insecticide type	Treatment	Y = a + bX	LC_{50}[a] ($mg\,L^{-1}$)	95% confidence interval ($mg\,L^{-1}$)	Correlation
Acetamiprid	Insecticide without Zn	$Y = -1.8 + 1.5X$	15.81	12.87–20.29	$R^2 = 0.96$
	Insecticide with Zn	$Y = -1.5 + 1.2X$	17.06	13.30–25.43	$R^2 = 0.91$
Imidacloprid	Insecticide without Zn	$Y = -1.0 + 6.4X$	38.88	24.16–74.93	$R^2 = 0.81$
	Insecticide with Zn	$Y = -1.1 + 0.7X$	36.78	20.33–105.23	$R^2 = 0.99$

[a] LC_{50} (Lethal Concentration 50, $mg\,L^{-1}$) represented drug concentration which killed 50% of the control object. It was commonly used to indicate the size of the acute toxicity.

3.3. *Toxicity evaluation of two insecticides with and without Zn*

The LC_{50} value representing the size of the acute toxicity and the LC_{50} is smaller, the bigger the toxicity (Laecke et al., 1995; Lydy and Linck, 2003; Rumpf et al., 1997). The LC_{50} value was very similar between two different treatments for each insecticide type (Table 4). For the Acetamiprid and Imidacloprid, the 95% confidence interval of insecticides with Zn addition contained the 95% confidence interval of insecticides without Zn, respectively (Table 4). There was a significant linear relationship between the concentration of insecticides and insecticide toxicity for each treatment (Table 4). These results indicate absence of any adverse interaction between Zn and insecticides regarding the toxic effect of the tested insecticides on insects used.

3.4. *Economic analysis*

The costs associated with the T2 and T3 applications were 8.1% and 0.9% higher than that of the T1 application, while the net income increased by 2.3% and 8.8% with the T2 and T3 treatments (Table 5). Compared with T2, the costs of T3 were reduced by 6.7% while the net income was increased by 6.9% (Table 5).

4. Discussion and conclusion

In the current study, the application strategy (i.e., Zn fertilizer together with insecticide or one day after insecticide application) did not affect the insecticidal effects of the insecticides used or Zn enrichment of grains with Zn by foliar Zn fertilizers in winter wheat. Foliar Zn application together with insecticides or afterwards increased grain yield significantly (Table 3), which is consistent with previous studies (Cakmak et al., 2010b; Zhang et al., 2012). During the wheat growing season, stresses such as drought conditions, high temperature and long sunny days are very common in NCP in China. During the reproductive growth stage, foliar Zn fertilizers can improve photosynthesis and pollen viability, phloem transportation of photoassimilates and antioxidative defense against oxygen free radicals generated under stress conditions such as drought and heat (Cakmak, 2000; Bagci et al., 2007 Bagci et al., 2007). In well agreement with his, a previous work from the same location showed that Zn foliar application could improve the resistance of winter wheat to drought (Karim et al., 2012). The current study shows that grain yield is improved by Zn foliar application at two locations and in combination with two insecticides (Table 1). This finding indicates that Zn foliar application is a highly adaptive approach (Zhang et al., 2012).

Zinc foliar application together with insecticide or afterwards resulted in significant increases in grain Zn concentration and content at two locations (Fig. 1). In previous reports, studies were conducted by applying the insecticide and Zn fertilizer separately. To our knowledge, no published reports available studied role of combined use of Zn fertilizer and insecticide in biofortification of wheat with Zn. In fact, Zn biofortification of wheat is very important for human health by alleviating Zn deficiency incidence in human populations (Cakmak, 2008; Zhang et al., 2012). Based on large surveys, the average grain Zn concentration of wheat ranged between 20 and 35 $mg\,kg^{-1}$ globally (Cakmak et al., 2010a; Zou et al., 2012; Rengel et al., 1999), which is below the daily human requirement. Grain Zn concentration should be increased to about 40–50 $mg\,kg^{-1}$ to have a measurable biological impact on human health (Ortiz-Monasterio et al., 2007). In the present study, average grain Zn concentration was increased to 57.3 $mg\,kg^{-1}$ by Zn foliar application applied together with insecticides or alone. This concentration achieves the targeted levels of Zn in wheat grain (Fig. 1) regardless of insecticide use and locations and is consistent with previous reports (Yilmaz et al., 1997; Cakmak et al., 2010b; Zhang et al., 2012 Zou et al., 2012).

Wheat aphid has a significant negative impact on wheat production (Rabbinge et al., 1981). Previously it has been reported that a yield loss of 7% occurs in wheat if the density of aphids per tiller was 10, and if the density of aphids per tiller increased from 10 to 40, then yield loss of wheat was reported to be 11%

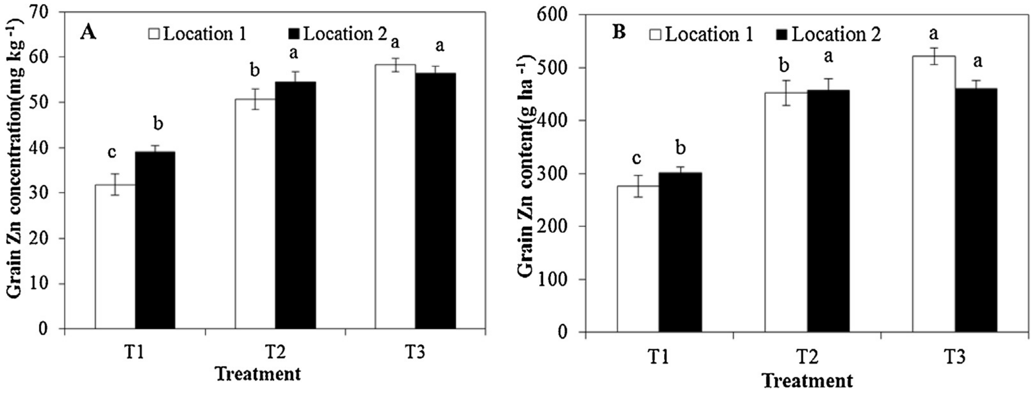

Fig. 1. Effects of insecticide treatment with or without Zn on grain Zn concentration (A) and content (B) of winter wheat. Mean values ($n = 8$) followed by different letters indicate significant differences at $P < 0.05$ by the LSD test. T1 represents the only insecticide applied treatment, T2 represents the foliar Zn fertilizer applied one day after insecticide application, T3 represents foliar Zn fertilizer applied together with insecticide.

Table 5
Economic analysis related to application approaches of insecticide with or without Zn treatments for winter wheat in the field.

Treatments	Price of grain (USD Mg^{-1})	Cost[a] (USD ha^{-1})	Net income (USD ha^{-1})	Income increase (USD ha^{-1})	Income increase (%)
T1	368	1663	1347 a[b]	–	–
T2	368	1798	1378 ab	31	2.3
T3	368	1678	1473 b	126	9.4

[a] USD 1 = 6.1 Chinese Yuan (2014 year).
[b] Values followed by different letters in each column indicate significant differences at $P \le 0.05$ by the LSD test. T1 represents the only insecticide applied treatment, T2 represents the foliar Zn fertilizer applied one day after insecticide application,T3 represents foliar Zn fertilizer applied together with insecticide.

(Shaoyou et al., 1986). In this study, there was also a concern about whether the foliar Zn fertilizer would affect the effectiveness of the insecticides. In the field study, no wheat aphids were recorded after insecticides were applied (data not presented). Therefore, we tested this issue in the laboratory. Insect body immersion method is one of main methods to evaluate the effects and environmental biosecurity of insecticides under laboratory conditions (Laecke et al., 1995), and LC_{50} is one of the important indices for the insecticides toxicity evaluation (Laecke et al., 1995; Lydy and Linck, 2003; Rumpf et al., 1997). In this study, the LC_{50} was very similar between insecticides applied alone and insecticides applied with $ZnSO_4{\bullet}7H_2O$ for each insecticide type (Table 4). The toxicity of the mixture of insecticide and $ZnSO_4{\bullet}7H_2O$ was not significantly different to the toxicity of the insecticides applied alone for two insecticides. This indicates that the foliar application of insecticides together with Zn does not affect the potency of insecticides.

Saving labor and production costs and increasing net income for farmers are very important considerations when a new agronomic approach is recommended. In this study, compared with Zn fertilizer applied one day after insecticide application, Zn foliar application together with insecticide reduced the labor costs (3 times) to 120 USD ha^{-1} (Table 5). As mentioned before, in China, insecticide spray is a common approach to control aphids in wheat production systems. If Zn biofortification of wheat is required, farmers should apply Zn fertilizer by the foliar approach again. Under this scenario labor costs are expected to be doubled. The results in Table 5 show that the Zn foliar spray together with insecticide could greatly solve this problem and save labor. Compared with foliar insecticide application only, the farmers' net income increased by 118 USD ha^{-1} due to insecticide and Zn foliar applications being made together (Table 5). As one of the primary wheat producing countries, the Chinese total cultivated wheat area is 24.3 million ha^{-1} (NBS, 2013). If this approach is widely adopted, the Chinese farmers' net income could be increased by 2867.4 million USD every year. More importantly, the farmers could improve their health while increasing revenue.

Therefore, $ZnSO_4$ can be mixed and sprayed together with insecticide without causing any adverse effect. The application time of zinc and insecticide is very important to improve the grain Zn concentration and control the wheat aphids. Cakmak et al. (2010a) reported that the suitable time of foliar Zn application for Zn biofortification of wheat was at booting + anthesis + early milk stages. And according to our field survey, those are the stages with the peak infestation of the wheat aphids. So to ensure the double effects, the suitable time of foliar Zn application and insecticide was booting + anthesis + early milk stages, and to improve the effect, the spray should be conducted after sunset in days without wind ($<0.2\ m\ s^{-1}$).

Finally, in this study, we presented an agronomic approach that is feasible economically beneficial and easily adoptable in many respects. Firstly, a combined foliar spray of insecticide with Zn did not cause any antagonistic reaction. Secondly, the timing of foliar Zn application and foliar insecticide is almost synchronous. Additionally, it could save labor and significantly increase farmers' profit. Finally, Zn biofortified wheat will, of course, also benefit health.

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