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# Relationship between potassium chloride suppression of corn stalk rot and soil microorganism characteristics

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**Abstract** Observations from a site-fixed field trial of 12 years in Jilin Province show that potassium chloride (KCl) application has a significant positive influence on corn stalk rot incidence. Incubation experiments were conducted to study the effects of KCl and soil extracts on the growth of *Fusarium graminearum*, the most common stalk rot fungi in this area, and the population changes in rhizosphere fungi, bacteria and actinomyces at different growth stages of corn. The results show that KCl addition to the potato dextrose agar (PDA) medium could not directly suppress *Fusarium graminearum* development. Soil extracts from soil samples taken from the field plots with and without KCl application affected *Fusarium graminearum* development, with soil extracts with KCl treatments suppressing *Fusarium graminearum* development more significantly, compared with that from the KCl-free treatment. These results indicate that soil extracts play a role in the interaction between corn and *Fusarium graminearum*. Long-term KCl application may increase the populations of rhizosphere fungi and actinomyces in the early growth stages, while there is no significant difference in the number of bacteria in rhizosphere among the treatments. Also, the populations of rhizosphere fungi are negatively correlated with the incidence of stalk rot in the early growth stages of corn. The sensitive infection stages of pathogen to corn consist of the stages when there is significant difference in the populations of rhizosphere fungi and actinomyces. The change of microorganism populations (especially fungi) in soil may be associated with the incidence decrease and is one of the mechanisms of KCl suppressing corn stalk rot.

**Keywords** corn, microflora, potassium chloride, rhizosphere, stalk rot

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## 1 Introduction

Stalk rot is one of the most persistent and destructive diseases of corn worldwide. Fusarium stalk rot is widespread throughout Jilin Province, which is the major corn growing area of China. As a kind of soil-borne fungal disease, corn stalk rot is difficult to control by conventional methods such as the use of synthetic fungicides and fungicidal seed treatment (Chen, 2000), (Munkvold G, 2002, Crown rot symptoms common in corn. [http://www.ent.iastate.edu/Ipm/Icm/2002/7-1-2002/crown\\_rot.htm](http://www.ent.iastate.edu/Ipm/Icm/2002/7-1-2002/crown_rot.htm)). Many studies showed that potassium application was an effective method to reduce the severity of corn stalk rot (Mei et al., 2003; Heckman, 1998; Li et al., 2004). Previous studies were mainly focused on the difference of defense mechanisms against stalk rot between susceptible and resistant varieties of corn; nevertheless, little information is available on the mechanism of suppression of corn stalk rot by potassium fertilizers (Yang et al., 2004; Long et al., 2003; Munkvold et al., 1997; Song et al., 1995). Many soil-borne diseases are influenced by rhizosphere soil microflora, but little attention has been paid to the effect of potassium on the soil microflora and its relation to corn stalk rot severity (Mcspadden and Weller, 2001; Elsas et al., 2002; Chen et al., 2005). Therefore, the studies of the effect of soil extracts on the growth of pathogen and the change of soil microflora after long-term potassium application are necessary in clarifying the suppression mechanism of potassium to corn stalk rot, and can provide a theoretical basis for ecological management of corn stalk rot.

## 2 Methods

### 2.1 Experimental design

The study was conducted in a long-term field experiment initiated in 1993 in Jilin Province. The treatments consisted of three levels of K [as potassium chloride (KCl)]: K0, K113 and K225 (Table 1), laid out in a randomized complete block design with four replicates. Each plot measured 40 m<sup>2</sup>. At the start of the experiment (April, 1993), the soil of

these experimental plots had the following characteristics: organic—C 22.4 g/kg, alkali—N 97.3 mg/kg, available—P 14.2 mg/kg, available—K 130.4 mg/kg. One-third of N, total P and K were applied as basal application, the remaining 2/3 N was applied as top application. N, P and K fertilizers used were urea,  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  and KCl. Soil samples were taken before experiment in 2004 and the soil available-K of K0, K113 and K225 treatments was 64.6, 86.8 and 122.8 mg/kg, respectively. Plant population for corn is 50 000 plants/hm<sup>2</sup>. Pingan38, a commercial corn hybrid, was applied in the experiment in 2004. The incidence of corn stalk rot was investigated before harvest (Li et al., 2001).

## 2.2 Effects of KCl nutrition and soil extracts on *Fusarium graminearum* growth

KCl with different concentrations (0, 20, 100, 500 and 1 000 mg/mL in K) was added to sterile potato dextrose agar (PDA). Plugs of inoculum (6 mm in diameter) were sectioned from the edges of five-day-old cultures of *Fusarium graminearum* with a cork borer and were placed onto the center of the Petri plates. Petri plates were incubated at 28°C. The diameter of the resultant fungal mycelium was measured three days after inoculation.

Soil samples (0–20 cm topsoil) were collected using multi-sampling method before seeding, with which the 1:1 (water/soil) soil extracts were collected. The soil extracts were filtered through a membrane filter with a pore size of 0.22 µm after being condensed, and then were added to sterile PDA. For controls, *Fusarium graminearum* was grown on PDA alone. Plugs of inoculum (6 mm in diameter) were sectioned from the edges of five-day-old cultures of *Fusarium graminearum* with a cork borer and were placed onto the center of the Petri plates. Petri plates were incubated at 28°C. The diameter of the resultant fungal mycelium was also measured three days after inoculation.

## 2.3 Rhizosphere soil sampling

Rhizosphere soil was sampled in the seedling, joining, tasseling, filling, milk and fully ripe stages of corn (18, 48, 66, 96, 116, 133 days after emergence, respectively). Detailed method for sampling from rhizosphere soil was as follows (Liu et al., 1997). Whole corn root system was carefully excavated so that it could be recovered and undamaged as much as possible (soil volume different with root itself). Then shake off the big soil bulk from roots, put the roots into a plastic bag, and shake the roots to collect the soil adhering to the surroundings of a root which is called “rhizosphere soil” in general. The collected soils were sieved in a 1 mm mesh with hair roots removed as much as possible. Fresh soil was used for the analysis of soil microflora.

## 2.4 Microorganism screening and enumeration

The number changes of microorganisms in rhizosphere were measured with the plate counting method (Xu and Zheng,

1986). The media used for the enumeration of bacteria, fungi and actinomyces were beef extract peptone medium, Martin agar medium and Gause’s No.1 synthetic medium, respectively. Each sample was replicated four times. The water content of the soil was determined with an aluminum box.

## 2.5 Data analysis

All data manipulation and statistical analysis were performed using Excel and SAS 8.2 for Windows. When a particular *F* test was significant, We compared means in the figures and tables using Fisher’s Least Significant differences (*LSD*) multiple comparison.

# 3 Results

## 3.1 Effects of potassium chloride application on stalk rot incidence of corn

The severity of corn stalk rot was significantly reduced by the application of KCl (Table 1). When the plants were supplied with 113 kg, 225 kg of K<sub>2</sub>O per hectare, the incidence of corn stalk rot was significantly decreased by 41.1% and 47.7%, respectively, in comparison with that without KCl application. But no significant difference was observed in the incidence of stalk rot and production between the K113 and K225 treatments. Statistical analysis showed that significant negative correlation was obtained between potassium chloride application rate and the incidence of corn stalk rot ( $r = -0.9233^{**}$ ,  $n = 12$ ). Low soil K levels can increase the risk for stalk rot and perhaps cause yield decreasing.

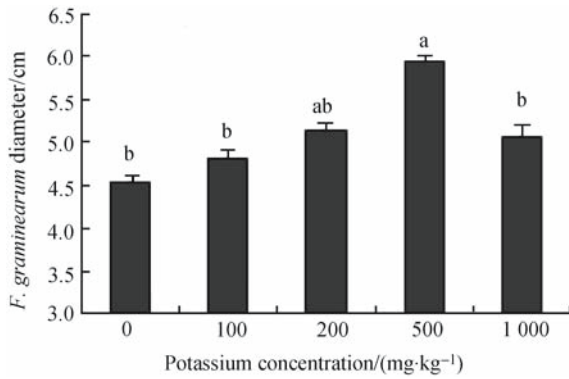
**Table 1** Effect of potassium chloride on corn stalk rot incidence and relative immunization efficiency

Treatment	Disease incidence/%	Relative immunization efficiency/%
K0	56.6a	–
K113	33.3b	41.1
K225	29.6b	47.7

Note: Different letters denote significant at  $P < 0.05$  level.

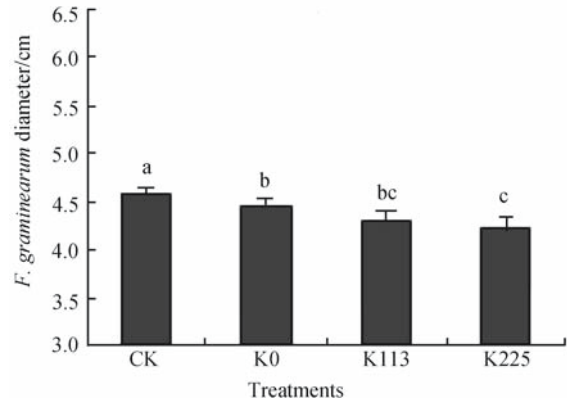
## 3.2 Effects of KCl nutrition and soil extracts on the growth of *Fusarium graminearum*

The pathogens of corn stalk rot have obvious regional characteristics. *Fusarium* stalk rot of corn, the most common corn stalk rot in Jilin Province, is caused by the fungus *Fusarium graminearum* (Jin et al., 1995). Therefore, the growth of *F. graminearum* affected by KCl and the soil extracts were studied in this experiment. As shown in Fig. 1, the higher the potassium concentration (0–500 mg/kg) in the PDA medium, the faster *F. graminearum* mycelium grew. When the potassium concentration in the PDA medium was higher than 1 000 mg/kg, which is far more than that in soil, the growth speed of *F. graminearum* slowed down but still faster



Note: Different letters above the square column denote different at  $P < 0.05$  level.

**Fig. 1** Effect of potassium chloride nutrition on *F. graminearum* development



Note: Different letters above the square column denote different at  $P < 0.05$  level.

**Fig. 2** Effect of soil extracts on *F. graminearum* development

than that with no KCl addition to PDA medium. The results suggested that KCl addition to the PDA medium could not suppress *F. graminearum* development directly. As can be seen from Fig. 2, the soil extracts from the treatments with no KCl and KCl application all inhibited the growth of *F. graminearum*. Soil extracts from soil of K113 and K225 treatments did not stimulate the growth of the mycelium with the increase of K concentration in soil extracts, but inhibited the development of *F. graminearum* mycelium more significantly, compared with that from soil of K0 treatment. From the results, we can speculate that soil extracts from long-term field trial must include other components that suppress *F. graminearum* development.

### 3.3 Effect of long-term potassium chloride application on soil microorganism flora

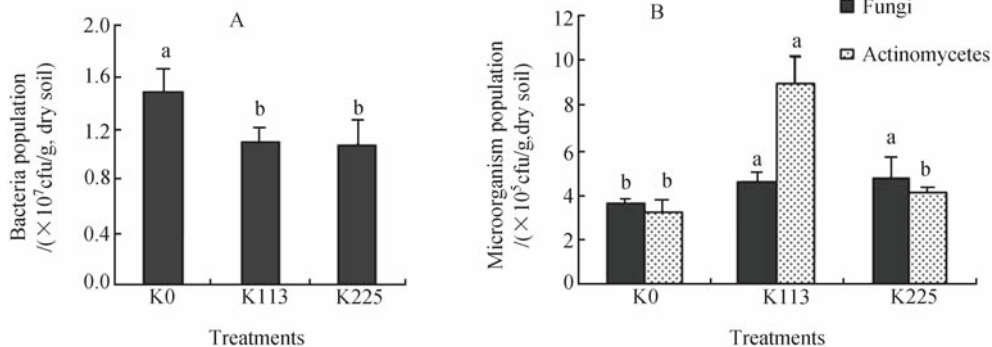
#### 3.3.1 Changes in the microorganism population before sowing

Long-term KCl application decreased the population of soil bacteria significantly before sowing. Compared with K0 treatment, K113 and K225 treatments reduced the population of soil bacteria by 26.2% and 27.5%, respectively. Long-term KCl application increased the population of soil fungi pronouncedly before sowing, and K113 and K225 increased the population of soil fungi by 28.8% and 31.8% respectively,

as compared to K0. No significant difference in the fungi population was observed between K113 and K225 treatments ( $P < 0.05$ ). The significant increase of actinomycetes population in soil was obtained in K113 treatment, and light increase was found in K225 treatment. Bacteria were the dominant flora in soil, the population of which was far more than fungi and actinomycetes in all treatments. The population of fungi was more than that of actinomycetes in soils with long-term no KCl and higher KCl application (Fig. 3). Statistical analysis showed that significant correlation was obtained between the fungi number before seedling and corn stalk rot incidence ( $r = -0.8154, n = 12, P < 0.01$ ).

#### 3.3.2 Changes in the fungi population in rhizosphere at various growth stages of corn

Table 2 shows the fluctuation of the fungi population in the growth stage of corn. At seedling, filling and fully ripe stages, the fungi population in rhizosphere in K113 treatment was more than that in K0 treatment significantly, but no pronounced difference was found at joining and tassel stages of the crop. At the joining, tassel and milk stages, the fungi population in the rhizosphere in K225 treatment was greater than that in the K0 treatment significantly, but no pronounced difference was found at the seedling and fully ripe stages. These results indicated that the fungi population in the rhizosphere of corn was increased to some different extent



**Fig. 3** Effect of long-term application of potassium chloride on microorganism population of soil

**Table 2** The fungi populations in rhizosphere of corn in different growth stages with different treatments ( $\times 10^5$ cfu /g DW)

Treatment	Seedling stage	Joining stage	Tassel stage	Filling stage	Milk stage	Fully ripe stage
K0	9.27b	5.91b	7.98b	8.12b	8.88a	6.40b
K113	11.63a	6.62ab	9.04ab	11.05a	8.71a	7.59a
K225	10.36ab	8.01a	13.71a	10.65a	8.22a	7.11ab

Note: Different letters denote significant at  $P < 0.05$  level (the same below).

after long-term KCl application except at the milk stage when no significant difference was observed in the populations of fungi among the treatments of K0, K113 and K225. After statistical analysis, significant correlation was obtained between the fungi numbers in the rhizosphere of corn at the seedling, joining, and tassel stages and the incidence of corn stalk rot, whose correlation coefficient was  $-0.5391^*$ ,  $-0.5868^*$ ,  $-0.6548^*$  ( $n = 12$ ), respectively.

### 3.3.3 Changes in the bacterial population in the rhizosphere at various growth stages of corn

As can be seen from Table 3, no significant difference existed in the bacterial populations in rhizosphere among the K0, K113 and K225 treatments at seedling, joining, tassel and filling stage of corn. At the milk stage, the rhizosphere bacterial population in K113 treatment was bigger than that in the K0 and K225 treatments significantly. However, as to the fully ripe stage, the bacterial population in the rhizosphere of corn with KCl application was less than that with no KCl application. These results indicated that the fungi population in the rhizosphere of corn was declined to some different extent after long-term KCl application except for the milk stage (Table 3).

**Table 3** The bacterial populations in the rhizosphere of corn in different growth stages with different treatments ( $\times 10^7$ cfu /g DW)

Treatment	Seedling stage	Joining stage	Tassel stage	Filling stage	Milk stage	Fully ripe stage
K0	9.04a	6.37a	15.12a	12.54a	10.36b	14.60a
K113	8.96a	7.36a	11.78a	9.78a	13.69a	7.11b
K225	8.94a	7.65a	13.19a	8.54a	7.11c	6.11b

### 3.3.4 Changes in the actinomyces population in the rhizosphere at various growth stages of corn

At seedling, joining and tassel stages, the actinomyces populations in the rhizosphere of corn in the K113 treatment were significantly greater than those in the K0 and K225 treatments (Table 4). In the whole growth course of corn, there was no significant difference between the treatments of K0 and K225 ( $P < 0.05$ ). At the filling and milk stages, the trend of the actinomyces amount was ordered  $K113 > K225 > K0$ , but there was no statistically significant difference among the treatments of K0, K113 and K225. As for fully ripe stage, significant difference was observed between K0 and K113, and the trend of the actinomyces

**Table 4** Actinomyces populations in the rhizosphere of corn in different growth stages with different treatments ( $\times 10^5$ cfu /g DW)

Treatment	Seedling stage	Joining stage	Tassel stage	Filling stage	Milk stage	Fully ripe stage
K0	69.10b	21.55b	23.58b	10.92a	10.43a	10.20b
K113	92.42a	33.17a	36.45a	12.40a	10.64a	13.81a
K225	74.44b	20.63b	20.48b	11.03a	11.09a	12.70ab

amount was still the order  $K113 > K225 > K0$ . The results suggested that long-term KCl application at the appropriate rate could increase the actinomyces population in the rhizosphere of corn to some different extent.

## 4 Discussion

### 4.1 Effects of KCl nutrition and soil extracts on *Fusarium graminearum* growth

It has been observed from this 12-year fixed site field trial that potassium chloride (KCl) application could reduce corn stalk rot incidence significantly. Dutta and Isaac (1979) summarized the possible mechanisms of inorganic amendments in reducing severity of soil-borne disease as follows: (1) by stimulating antagonistic micro-organisms in the rhizosphere and in turn inhibiting the pathogen; (2) by inhibiting the pathogen directly; (3) by changing the host metabolism and creating an unfavorable environment within the plant and at the root part; (4) control by combined effect, i.e., by changing the host metabolisms, inhibiting the pathogen directly and by stimulating antagonistic microflora in the rhizosphere. The results in this experiment indicated that KCl could not inhibit the growth of *Fusarium graminearum* directly. The soil extracts from the soil with long-term KCl application and no KCl application all inhibited the growth of *Fusarium graminearum* pronouncedly, and soil extracts from soil with long-term KCl application inhibited the pathogen growth more significantly than that with no KCl application, which could result from two aspects—on the one hand, changes of the content of other elements caused by long-term KCl application; on the other hand, changes in the content and component of corn root exudates. Root exudates obtained from a tolerant variety of corn inhibited the growth of *Sporisorium reilianum* f. sp. Zeae., and the proliferation of the yeast form, and indicated that some molecules in root exudates could play a role in the pre-infectious stage between corn and *S. reilianum* f. sp. zeae. (Martinez et al., 2001). Therefore, there was a close relationship between the root exudates affected by long-term KCl application and the decline of infection probability needs to be further investigated.

### 4.2 Effect of long-term application of KCl on soil microflora and its relation with the infection of pathogen

The results obtained from the study of long-term experiment in red soil indicated that long-term KCl application did not

influence the bacterial number significantly, but increased the number of fungi and actinomycetes (Xu et al., 2003). Belay et al. (2002) reported that the number of fungi in soil with long-term N, P and K application was significantly higher than that in the other treatments, but the numbers of bacteria and actinomycetes did not differ significantly in the treatments between N, P and K application and no fertilizer application. This result observed from this 12-year fixed site field trial was not coincident absolutely with the previous reports, but they were in common that the fungi population in soil and the rhizosphere increased after long-term KCl application. Long-term KCl application at a proper rate may increase the actinomycetes amounts significantly, and which was declined greatly with further increase of application rate. Xue et al. (1995) analyzed the effect of long-term application chloride-containing fertilizers on the microorganism flora, and found that higher chloride application rate inhibited the multiplication of actinomycetes. Therefore, chloride in KCl also played a role in the change of microorganism flora.

Many studies only focused on the effects of long-term fertilization on the soil microflora in non-rhizosphere, but paid little attention to the change of microorganism populations in the rhizosphere. Our research further analyzed the changes of bacteria, fungi and actinomycetes populations in different growth stages of corn, and found that long-term KCl fertilization had no significant effect on bacterial population at early growth stages, but increased the number of fungi significantly. The change trend of the number of microorganisms at the early growth stages differed from that at the latter growth stages of corn. A significant negative correlation was obtained between the incidence of corn stalk rot and the number of fungi at its early growth stages, but no significant correlation existed between the incidence of stalk rot and fungi number or actinomycetes number. The best linear correlation relation was obtained between soil fungi number before seedling and corn stalk rot incidence. The study of Li et al. (1998) about *Vorticillium dahliae* (soil-born disease) showed that the rhizosphere fungi and actinomycetes population of resistance cotton cultivars was more obvious than susceptible ones, and that there was no close correlation between the rhizosphere bacteria population and the cotton resistance to *Vorticillium dahliae*. Therefore, the change in the populations of soil microorganisms (especially fungi) caused by long-term KCl application may be associated with the decline in the incidence of corn stalk rot.

The difference of fungi number was responsible for the difference of resistance of soil to *Rhizoctonia solani* in potato (Höper and Alabouvette, 1996). The suppression of soil resulted from the competition of different microbial groups. The mechanisms include that the competition for resources between the total microbial biomass and *Fusarium* spp., and that the competition between the pathogen *Fusarium* and non-pathogen *Fusarium* in soil (Larkin et al., 1996; Larkin and Fravel, 2002). Fertilization may influence the total microbial biomass and components, especially fungi groups

(Jia et al., 2004). The number and rate of fungi inhibiting *V. dahliae* in resistant cultivars are more than in susceptible ones (Li et al., 1999). Therefore, in addition to the total microbial biomass, the microbial components, dominating groups and their inhibition to pathogen are all responsible for the real incidence of soil-born diseases. In this study, we found that *Trichoderma* was the dominating fungus in the rhizosphere of corn with KCl application. The increase of fungi population may be caused by the increase in amounts of some specific fungi group. Therefore, it is necessary to analyze the number of various fungi components to make clear the mechanism of KCl suppression of corn stalk rot.

The infection at early growth stages of corn was considered to be dominated over the cycle of the stalk rot (Chen et al., 1995). In this study, the sensitive infection stages (early growth stages of corn) of pathogen to corn consisted of the stages when the significant difference existed in the rhizosphere fungi and actinomyces populations, and no significant difference was found in bacterial population. The negative correlation was observed between the fungi numbers at the sensitive infection stages and the incidence of corn stalk rot. Therefore, the changes of microflora in the rhizosphere may be associated with the infection of pathogen to corn root closely.

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