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Full Length Research Paper

Allelopathic influence of leek (*Allium porrum*) seeds on germination and radical growth of flue-cured tobacco of different cultivars

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Leeks (*Allium porrum* L.) are increasingly included in the rotation or inter-planting with tobacco in many provinces of China and worldwide. Therefore, we examined the possible allelopathic potential of leek seeds on the germination, seedling emergence and root elongation of flue-cured tobacco of different cultivars. The effects of imbibed leek seeds, their aqueous leachates and extracts of the seed on the germination and root elongation of the seeds of four test flue-cured tobacco cultivars, K 326, Yunyan 85, Zhongyan 100 and NC 89, under both aseptic and soil conditions, were investigated. Inhibitory effects were evident in all of the bioassays of these tobacco seeds. The leachates of the leek seeds and the aqueous seed extract were highly inhibitory, but the seeds themselves were less inhibitory. The observations indicate a release of inhibitory substances from the leek seeds leading to the observed inhibitory effects. The results of this study may be very useful for the management of crop rotations/systems involving leek as a component.

Key words: *Allium porrum* L., allelopathy, flue-cured tobacco, inhibitory effects, seed germination, seed extract.

INTRODUCTION

Tobacco is an important economic crop in China and many other countries worldwide (Liu, 2003). It is subjected to damages by diseases and pests (Zhu et al., 2002; Lai and You, 2010; Lai and Lai, 2010; Lai et al., 2011a, 2011b). Except for the allelopathic effects of garlic on flue-cured tobacco of different cultivars (Lai et al., 2010), no reports of such effects on tobacco and other plants or crops were found in the available literature. Allelopathy refers to the phenomenon of a direct or indirect chemical-mediated influence of one plant species on another in their shared environment. It has been proposed that this adaptive mechanism (Kong, 1998; Suman et al., 2002), and the germination of seeds and the growth of seedlings are often inhibited or promoted by allelopathic compounds of the living or dead tissue of another plant in the vicinity (Jaseep, 1990; Wardle et al., 1991; Sogaard and Doll, 1992; Van and Grobbelaar, 1995; Laterra and Bazzalo, 1999; Kong and Hu, 2001; Mo et al., 2004; Dong et al., 2005; Zhou and Chen, 2007). However, modern agriculture aims at maximizing land use to grow crops in rotation through relay and intercropping systems where the co-occurrence of the seeds (or seeds and seedlings) of more than one species in the same bio-niche is necessary (Mou and Lei, 1999; Suman et al., 2002). Currently, techniques of mixture, relay and inter-planting are employed to protect crops from pests and/or diseases (Zhu et al., 2000; You et al., 2007; Shen et al., 2007; Cai

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et al., 2007, 2011; Lai et al., 2011a, 2011b; Lin et al., 2011). However, information of counter or synergistic allelopathy is necessary to establish an effective system of cultivation, especially those of relay and intercropping systems (Mou and Lei, 1999; Suman et al., 2002). Leeks (*Allium porrum* L.) are widely planted in China, including the provinces of Fujian, Guangxi, Guizhou, Chekiang, Hubei, Hunan and Shanxi, and several other countries worldwide (Ren, 2003). In the aforementioned districts, it is common for the farmers to also plant tobacco in rotation or inter-planting. However, the allelopathic effects of leeks on tobacco have not been extensively reported.

The present investigation was initiated to examine whether leeks influenced flue-cured tobacco seeds and seedlings. The main objective was to assess the different allelopathic influences of leek seeds (*A. porrum* L.) on the germination, emergence and root-elongation of flue-cured tobacco of the different cultivars that are grown with leeks in various rotations.

MATERIALS AND METHODS

The experiments were conducted at the Longyan Substation of Fujian Science and Research Institute of Tobacco Farming in China from March to June in both 2008 and 2009. We used four cultivars of tobacco in the bioassays. The K 326 and Yunyan 85 flue-cured tobacco seeds used in the bioassays were obtained from the Longyan Substation of Fujian Science and Research Institute of Tobacco Farming in Longyan, China. The Zhongyan 100 flue-cured tobacco seeds were provided by the China Nation Tobacco Breeding (North) Center in Qingdao, China. The NC 89 flue-cured tobacco seeds were obtained from the China Nation Tobacco Breeding (South) Center in Yuxi, China. The *A. porrum* L. seeds were obtained from the Vegetable Corporation of Longyan, China. All of the experiment treatments in this study were arranged in a completely randomized design (CRD).

Direct seed assays

The bioassays were conducted to evaluate the effect of leek seeds on the germination and early root growth of flue-cured tobacco seeds, K 326, Yunyan 85, Zhongyan 100 and NC 89. Leek and cultivars of flue-cured tobacco seeds were surface-sterilized with a 1% sodium hypochlorite solution for 10 min and rinsed with sterile distilled water. The experimental units consisted of 20 seeds of each test tobacco cultivar placed uniformly along with different densities of leek seeds (0, 12, 24 or 48) under aseptic conditions on two layers of filter paper in a Petri-dish (12 cm diameter) moistened with 3 ml of distilled water. The dish was then sealed with a plastic film. The tested tobacco seeds were placed at regular spacing using a grid, and the leek seeds were placed regularly between the tobacco seeds and vice versa as the controls. Additional controls of only one type of seed arranged in this fashion were used to assess any self-allelopathic influence. Each treatment was replicated three times, and the Petri dishes were placed randomly within a growth chamber with a day/night temperature of 24/15°C and a 16 h photoperiod. Water was replenished as necessary (6 to 7 drops of distilled water per dish) to keep the filter paper saturated to avoid competition for water during germination (Suman et al., 2002). Seedlings with radicals protruding at least 1 mm through the seed coat were considered to be germinated.

The germination of all of the seeds was recorded on the 2nd, 4th, 6th, 8th, 10th, 12th, 14th and 16th day after the initiation of the experiment. The root length of four randomly chosen tobacco seedlings was measured in each Petri dish. Each experiment was replicated twice.

Seed leachate assays

The germination and root elongation of the K 326, Yunyan 85, Zhongyan 100, NC 89 and leek (as self-control) seeds were evaluated for the influence by the water leachate of the leek seeds. The leachate was prepared from 20 g leek whole seeds soaked in 200 ml of distilled water in a 500 ml flask for 24 h with shaking (SHZ-C, Shanghai Hede Experiment and Facility Limited Corporation) at 110 rpm and 22°C. The decanted aqueous solution was filtered through Whatman No. 2 filter paper and then passed through a 0.22-µm cellulose-acetate filter (Sartorius 11407-047-N). Twenty surface sterilized seeds of either K 326, Yunyan 85, Zhongyan 100, NC 89 or leek were placed separately in a sterilized bioassay system as described previously. The filter paper in each Petri dish was moistened with 3 ml of test solution or 3 ml of distilled water. Each treatment was replicated 3 times, and all of the Petri dishes were incubated under the same conditions as described earlier (Suman et al., 2002). The germination rate and radical lengths of four germinated seeds from each replicate were measured at 2-day intervals for 16 days.

Seed extract assays

The germination and root lengths of the K 326, Yunyan 85, Zhongyan 100 and NC 89 seeds in response to the whole extract of the leek seeds were also measured. For this, 20 g samples of leek seeds were ground to a powder in a Sample Mill (CSM-1, Shanghai Hede experiment and facility limited corporation) and soaked in 200 ml of distilled water in a 500-ml flask for 24 h with shaking (SHZ-C, Shanghai Hede experiment and facility limited corporation) and the experiment was performed as described for the seed leachate assays (Suman et al., 2002).

In-soil assays

The germination of all of the four test cultivars in response to the leek seeds and to the addition of the leachate of the leek seeds was evaluated using sieved soil from the Longyan Substation of Fujian Science and Research Institute of Tobacco Farming as the substrate. Seeds of K 326, Yunyan 85, Zhongyan 100, and NC 89 were sown in plastic pots (7 cm diam × 8 cm ht) and treated with one of the following: i) 5 ml of leek-seed leachate, ii) the equivalent number of leek seeds, or iii) 5 ml of distilled water as a control. Before sowing, the soil was saturated with tap water. After sowing (20 seeds per pot), the leek leachate (first treatment) or water (third treatment) was uniformly poured into the soil, and each pot was covered with a plastic film. For the second treatment, the leek seeds were uniformly co-distributed with the test cultivar seeds. Five pots per treatment were randomly placed in a greenhouse with 23/17°C-day/night temperatures, under a natural photoperiod (Suman et al., 2002). The emergence of the tobacco seedlings was counted after 12 days; after 25 days of the initiation of the experiment, the radical and above-ground height of eight seedlings from each replicate was measured.

Statistical analysis

An analysis of variance (ANOVA) and linear regression for a completely randomized design were used to compare the effect of

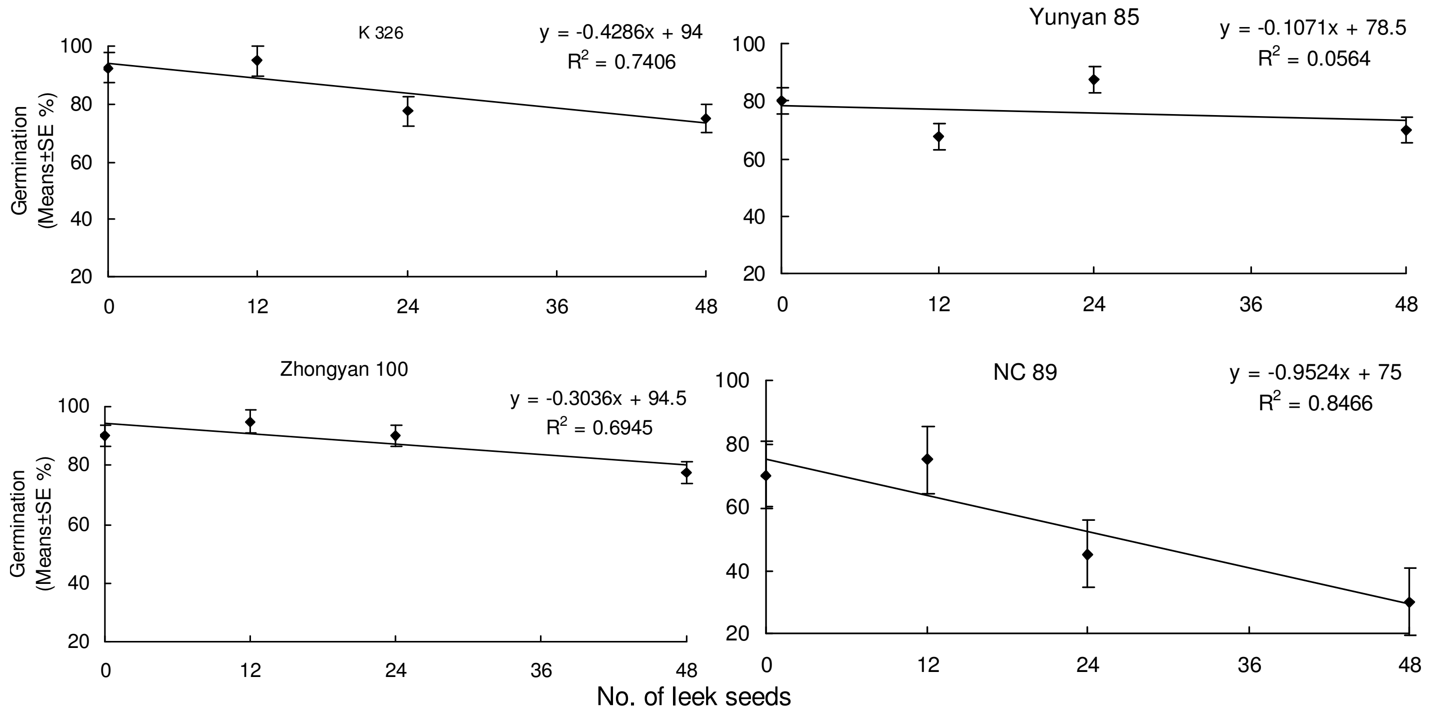

Figure 1. Effects of leek seed density on the germination percentage of tobacco seeds (20 each). Data are means ± SE.

the treatments on the seed germination and root elongation using DPS software (Tang and Feng, 1997). The treatment effects were considered significant when *P*<0.05.

RESULTS AND DISCUSSION

Influence of neighboring leek seeds

Due to the size of leek and tobacco seeds, the density of intercropping or relay systems and the fixed number of rotations per year tend to vary, the bioassays were performed using different numbers (0, 12, 24, 48) of tobacco seeds and a fixed number (20) of tobacco cultivars. In all cases, the seed germination and early seedling growth of the four tobacco cultivars were significantly inhibited by the presence of the leek seeds. Furthermore, the inhibitory effects were more severe as the densities of the leek seeds were increased (Figures 1 and 2). The strongest negative effect was observed in the case of NC 89 and the least with Yunyan 85 (germination) and K 326 (root length). The expansion of the cotyledons and root elongation of all of the cultivars were also adversely affected by the leek seeds; any ungerminated seeds of the tobacco cultivars were found to be unviable. In the control consisting of the reverse arrangement, the leek seed germination was 95% in the presence of the tested tobacco seeds. These results suggest that the allelopathic substances released during the imbibition of leek seeds have an inhibitory effect on the germination of the tested tobacco cultivars. Similarly, Kushima et al. (1998) have demonstrated that, upon imbibition, watermelon seeds release allelopathic substances that affect the germination and root growth of other crops, including amaranth, barnyard grass, cockscomb, lettuce and tomato (Kushima et al., 1998). Goldberg and Werner (1983) proposed the use of a target-neighbor design in which varying densities of a neighbor species are planted around a target plant for studying plant competition and allelopathic interactions (Goldberg and Werner, 1983). At high target plant densities, the toxin is shared (and, thus, diluted) among many plants, such that each receives a proportionally smaller dose (Weidenhamer et al., 1989; Weidenhamer, 1996). Thijs et al. (1994) have demonstrated the killing effect of atrazine on broad-leafed soybean in the presence of different densities of corn. The increased growth rate of soybean at the higher corn densities was mainly due to the uptake of atrazine by the corn plants and, hence, the dilution of the toxin present in the environment (Thijs et al., 1994).

By inference, a lower density of test seeds than those used in the bioassays would, therefore, be rendered more susceptible to leek allelopathy than that observed.

Influence of the water leachate and extracts of leek seeds

The germination and early seedling root length of all of the tested tobacco seeds were significantly inhibited by the leachate of the leek seeds for all the cultivars except

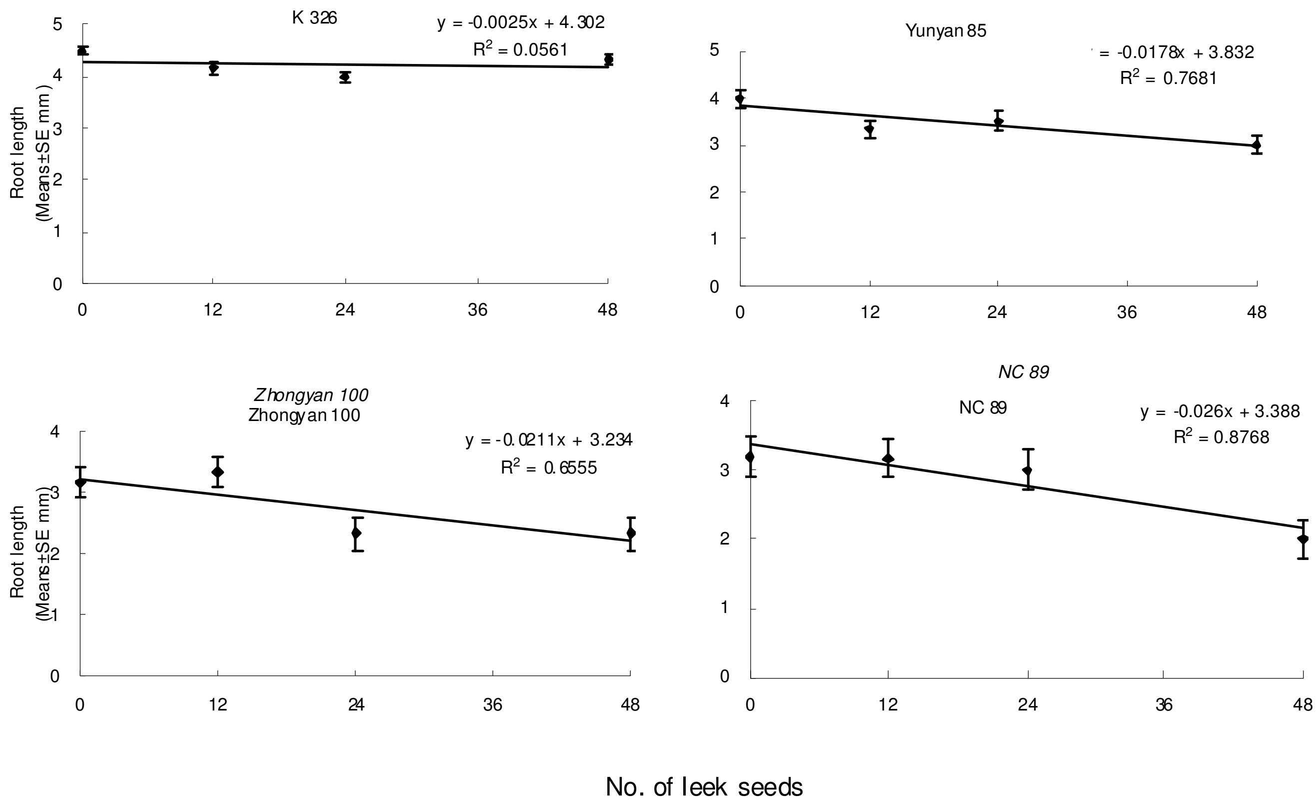

Figure 2. Effects of leek seed density on the root length of tobacco seedlings (20 each). Data are means ± SE.

Zhongyan 100 (Figures 3 and 4). This was in contrast to the short-term effect demonstrated by the allelopathic interactions of the weed species, *Lotes tenuis*, on *Cardus acanthoides* (Laterra and Bazzalo, 1999). However, our results were in agreement with the reported long-term effects of extracts of *Sesbania punicea* (Cav.) Benth seeds on the seedling development of a number of plant species (Van and Grobbelaar, 1995). However, we noted that the leek seed leachate also marginally inhibited the germination of its own seeds (7.4%), suggesting autopathy. Unexpectedly, in our study, the effects of the leek seed extracts on germination and root-development were greater than the effect of the leachate (Figures 3 and 4), which was not consistent with the results of Suman et al. (2002) who reported that the effect of a black-gram seed extract on germination and root-development was less, as compared to the effect of the leachate. This difference may be due to the flue-cured tobacco being more susceptible to an extract of the leek seeds than a leachate of them in terms of the allelopathic concentration of one or more inhibitory substances in the extract of leek seeds.

Influence of leek seeds and their leachate in the soil

When bioassayed on a soil substrate, the comparative emergence of the tobacco seeds showed a significant variation between the treatments. The presence of germinating leek seeds and also their extract significantly reduced the final percentage of germinated tobacco seeds and the root lengths and above-ground lengths of the seedlings ($P<0.05$) (Figures 5 and 6). In particular, a stronger inhibition of the germination, root length and above-ground length of the tobacco seedlings was found for the extract than for the leachate in the soil, except for Zhongyan 100. This result is inconsistent with Suman et al. (2002) who found that a weaker inhibition of the root length with extracts versus leachate in the soil (Suman et al., 2002). This difference may be attributed to an increased amount of substances in the leek seed extract than in the leachate of the seeds. Taken together, the results showed that the leek (*A. porrum* L.) seeds had an inhibitory effect on the seed germination and seedling growth of the tested tobacco cultivars and that the responses to the allelopathy were species-specific for the flue-cured tobacco seeds of different cultivars. The presence of a high density of leek seeds may also reduce the germination and slow the root development of other tobacco cultivars, consistent with Lola et al. (2004) and Lai et al. (2010). Lola et al. (2004) indicated that *Allium ursinum* inhibited seed germination and plant growth of other herbaceous plants (Lola et al., 2004), and Lai et al. (2010) found that the germination and growth of flue-cured

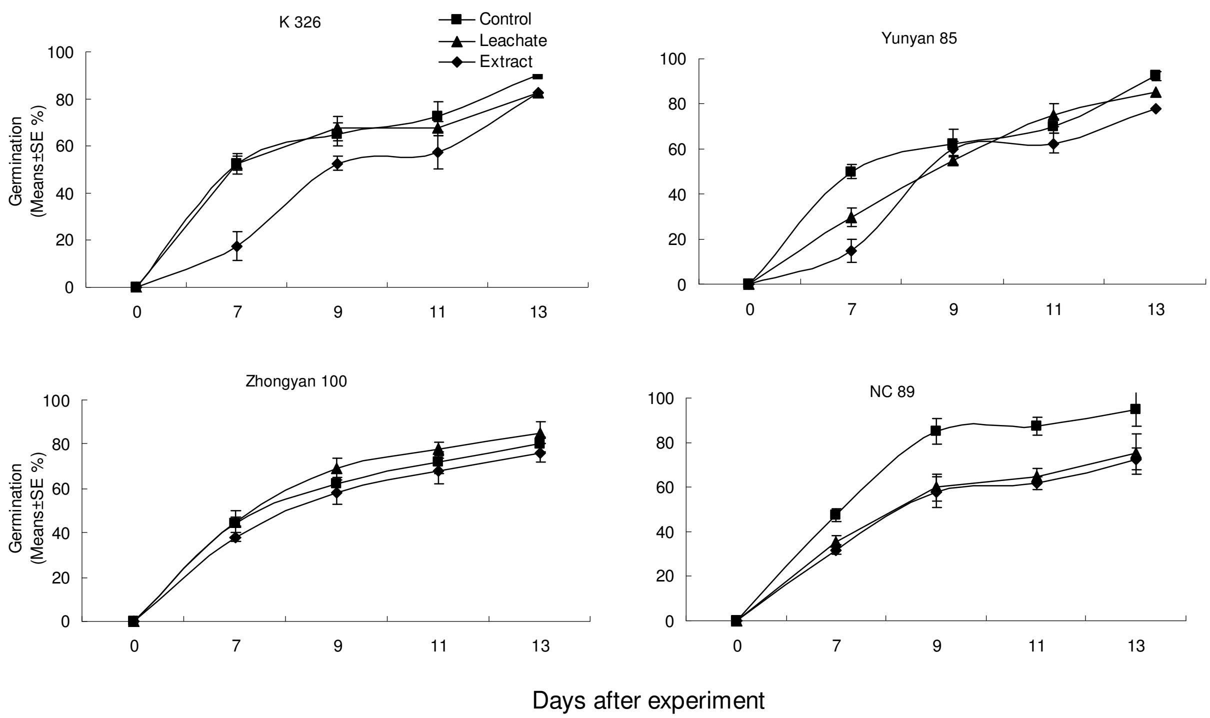

Figure 3. Effects of leek seed leachate and seed extracts on the germination percentage of tobacco seeds (20 each). Data are means ± SE.

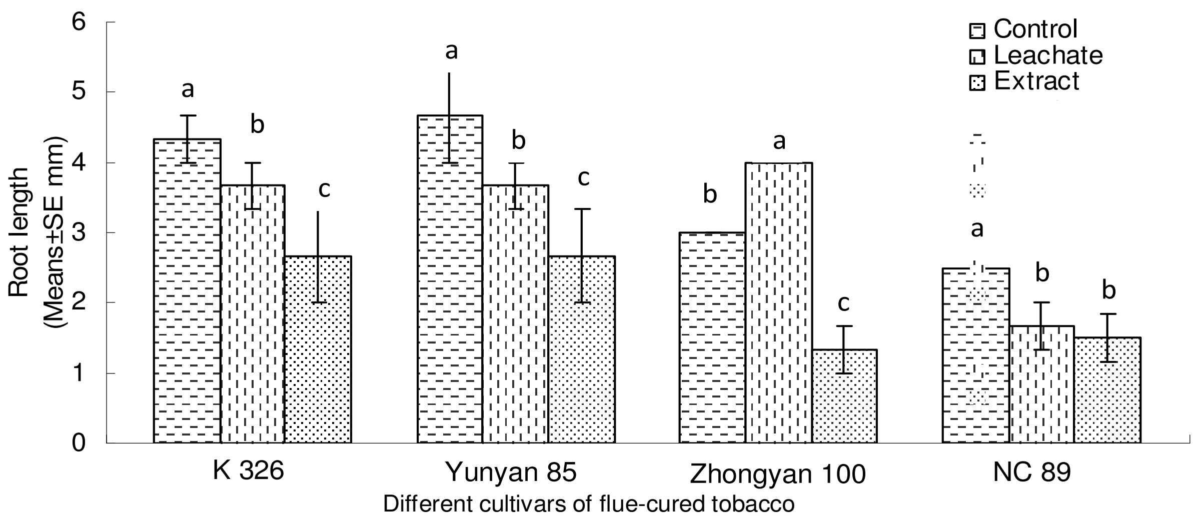

Figure 4. Effects of leek seed leachate and seed extracts on the root length of tobacco seedlings (20 each). Data are means ± SE. Means marked by the same letters were not significantly different at a 5% level of significance, as determined by ANOVA and Duncan's test.

tobacco seeds of different cultivars were also affected by garlic. The identification of methods to counteract these allelopathic effects of leek extracts may be very useful for the management of crop rotations/systems involving leek as a component.

Accordingly, research to identify such approaches, for example, the use of microbes is in progress.

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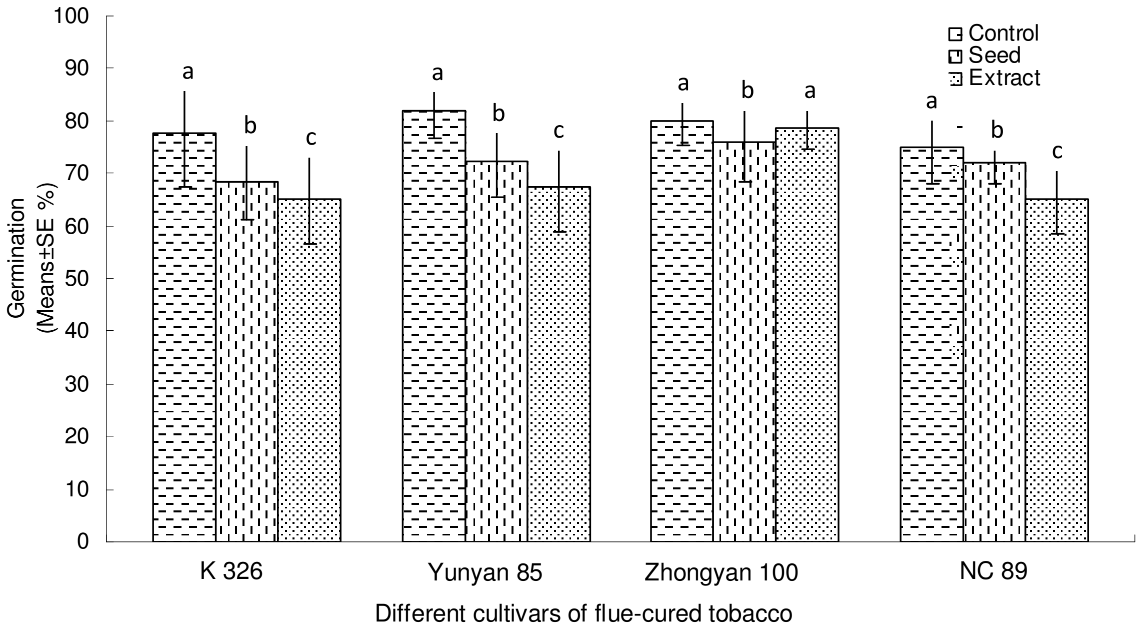

Figure 5. Effects of leek seeds and seed leachate on the germination percentage of tobacco seeds in soil (20 each). Data are means ± SE. Means marked by the same letters were not significantly different at a 5% level of significance as determined by ANOVA and Duncan's test.

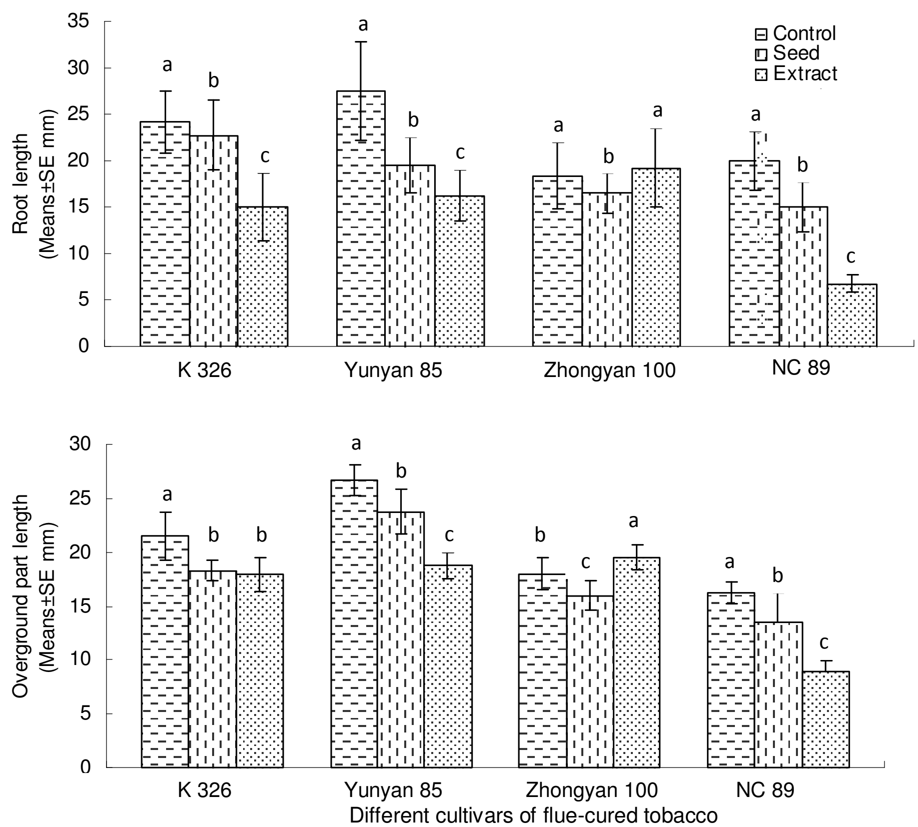

Figure 6. Effects of leek seeds and seed leachate on the root length of tobacco seedlings and above-ground length in soil (20 each). Data are means ± SE. Means marked by the same letters were not significantly different at a 5% level of significance, as determined by ANOVA and Duncan's test.

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