https://doi.org/10.26651/allelo.j/2021-54-2-1355 0971-4693/94 Euro 20.00
Allelopathy Journal 54 (2): 157-168 (Nov, 2021) *International Allelopathy Foundation* 2021
Table: -, Figs : 11

Allelopathic effects of Proso millet (*Panicum miliaceum* L) extracts on seed germination and seedling growth of Proso millet and maize

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(Received in revised form: May 28, 2021)

ABSTRACT

We evaluated the allelopathic effects of different concentrations of shoot, root and biomass (shoot + root) extracts of pure and intercropped Proso millet (*Panicum miliaceum* L., var. Mizao 52) on seed germination and seedling growth of maize (*Zea mays* var. Jinkkai No.3), and Proso millet in greenhouse experiment. The millet residue extracts decreased the seed germination and seedlings growth (radicle length and plumule length) of test crops than control. The shoot, root, and biomass residue extract of proso millet inhibited the seed germination and seedlings' growth of the recipient crops. Therefore, sowing maize and millet on fields after previous proso millet should be avoided.

Keywords: Allelopathy, inhibition, intercropping, maize, *Panicum miliaceum*, Proso millet, residues, seed germination, seedling growth, *Zea mays*

INTRODUCTION

Allelopathy is an interference mechanism where plants release chemicals that affects the other plant's growth, population and communities (9,24). Allelopathy plays an important role in the interaction of crops with another crop, crops with weeds, and weeds with another weed (5). Allelochemicals may stimulate or inhibit the growth of another plant; moreover, allelopathy is an environmentally friendly method for weed control (41). There are many ways to control weeds, including mechanical methods and farming methods; synthetic herbicides are still the most common method, however, the largescale use of synthetic herbicides is problematic, because there have been 502 cases of weed resistance and health and environment worldwide (35). The allelopathic potential of plants depends on the biomass, plant density, solubility and adsorption of allelochemicals in soil (42). The living and dead tissues (leaves, roots, stems, flowers) of plants during their decomposition release allelochemicals which cause allelopathic effects (7,19), thereby suppress the growth of neighboring plants (13,25,45). The decomposition of plant residues increases the soil microbial activity; if legume crops residues are decomposed, they release nitrogenous compounds, promoting the next crop's growth (22,42). Many crops and their residues affect the seed germination and seedlings'

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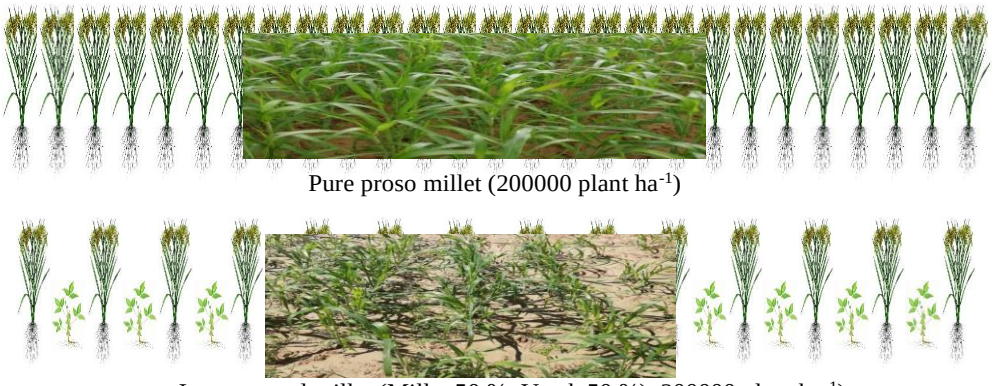

Pure proso millet (200000 plant ha^{-1})

Intercropped millet (Millet 50 %: Vetch 50 %), 200000 plant ha^{-1})

Figure 1. Pure and intercropped proso millet (millet-vetch) relay-strip intercropping system. "25 cm + 25 cm" one row of millet and one row of vetch.

Proso millet *(Panicum miliaceum L)* Maize *(Zea mays)*

Figure 2. The recipient plant seeds (proso millet, maize).

growth of other crops through the release of allelochemicals (14,17,26,29,38,40). The allelopathic interactions also occur between the crops in crop rotations (6). Many allelochemicals have been identified from the plants (33,43). The continuous monoculture leads to the accumulation of phytotoxins and harmful microorganisms in the soil, thereby causing phytotoxicity and soil sickness (31). Therefore, crop rotation is an option to minimize the adverse effects of monoculture, but subsequent crops may be negatively affected by phytotoxins released by earlier crops (12). Nonetheless, by breaking the cycle that may be harmful to the long-term management of a particular field, the rotation of selected crops can help protect (12). Therefore, weed-inhibiting crop rotation is an effective farming method that can control weeds without using herbicides (28).

Proso millet (*Panicum miliaceum* L.) ranks sixth among the world's important cereals (8). Its seeds are nutritious, hence, used as food and animal feed in China, Japan, India, and African countries, especially in the arid regions (2,8,38,27). About 20-varieties of proso millet are grown worldwide (20,44). Application of Proso millet residues in wheat

crop fields controls annual grass weeds, insect pests and diseases (11,38). In winter crops, sown after millets, the weeds seed bank is reduced up to 90 % (3,4,18,36). The Proso millet yield was decreased 9.6 % in monoculture or no-till conditions than grown in crop rotation with bean (23). The decline in Proso millet yield in monoculture due to autotoxicity is not known. Under field conditions, the release of allelochemicals from decomposing crop residues inhibits the seed germination, growth, and soybean yield (1,39). The stubble extracts of Proso millet inhibits the seed germination and seedling growth of wheat (15,35).

Proso millet allelopathic potential is known (21,46). However, the effects of its allelochemicals on subsequent crops germination and growth have not been investigated. Hence, this study aimed to evaluate the allelopathic effects of Proso millet residues extract on seed germination and seedling growth of *Panicum miliaceum* L. and *Zea mays* L.

MATERIALS AND METHODS

The study was conducted in a greenhouse at Yuzhong Grassland Agricultural Station, Lanzhou University, Yuzhong, Gansu Provence, China. [35.94 °N, 104.15 °E, Elevation : 1875 m]. The area has a semi-arid to arid continental climate, with hot summer (22.4 °C) and cold freezing winter (-5.3 °C), the mean annual rainfall: 315 mm, Rainy season: May to October. The winter is dry, with very little snow.

I. Extract preparation

The Proso millet biomass (roots, shoots) was collected after the harvest of field experiments in 2017 and 2018 from (i) pure millet (100 % millet) and (ii) millet intercropped with common vetch (50 % millet) (Figure 1). The biomass was air-dried in the shade at 30 ºC for ten days. The dry materials were grounded and passed through a sieve (1 mm in size). To prepare the extract, pure millet [80 g shoot, 40 g root and 110 g biomass (shoots + root)], while in intercropped millet, it was 40 g shoot, 15 g root and 55 g biomass, respectively. The above quantity of powdered plant material was separately soaked in 1000 ml distilled water at room temperature for 24 h and then filtered through Whatman No. 3 filter paper. The different concentrations (%) of proso millet extracts were: pure crop shoot (8 %), pure crop root (3 %), pure crop biomass (11 %), intercropped crop shoot (4 %), intercropped crop root (1.5 %) and intercropped crop biomass (5.5 %), respectively. The extract was stored as a stock solution in a refrigerator at 4 ºC. Distilled water was used as control. The extract was stored as a stock solution in a refrigerator at 4 ºC.

Petriplate bioassay

The experimental treatments consisted of 2 factors: (i). Proso millets 2 (Pure, intercropped) (Figure 1), (ii). Proso millet parts 3 (shoot, root, biomass), (iii). Recipient crops 2 [Maize (*Zea mays*) and Proso millet (*Panicum miliaceum* L)] (Figure 2). The treatments were replicated 10 times in Complete Randomised Design. Ten seeds of each recipient species were sown in each sterilized petri dish (9 cm dia) lined with two Whatman No. 3 filter papers. Ten ml Proso millet extracts of different concentrations were added to each petri dish at the beginning with pipettes. To keep

petri dishes moist, 5 ml extracts were added per petri dish on alternate days. The Petri dishes were kept in an incubator at 25 ºC in the dark. Seeds were considered germinated when 1 mm radicle was visible. The numbers of seeds germinated were counted daily till 15 days.

The following seeds germination parameters were determined

Seed germination: The seed germination was calculated as per (23,44) as under:

$$\text{Seed germination index} = \frac{\text{No. of germination seed}}{\text{Days of the first count}} + \ldots + \frac{\text{No. of germination seed}}{\text{Days of the final count}}$$

At the end of 15 days, the numbers of dead seeds were calculated as the difference between the total number of germinated seeds and the initial seed number sown.

Mean germination time

$$\text{Mean germination time (day)} = \Sigma n.D/\Sigma n$$

Where, n: Seeds germinated on day D. The lowest the mean germination time, the faster number of seeds sprouted (30).

Radicle and plumule length

A vernier caliper was used to measure the radicle and plumule length on 15th day after the germination phase. The inhibitory/Stimulatory (%) effects of extracts on seeds germination and seedlings growth of recipient plants were calculated as per Zhang and Fu (45). The results of extract-treated seeds were statistically compared with the control.

II. Statistical analysis

Data were subjected to the analysis of variance (ANOVA) using the SAS 9.4 software package. The ANOVA was performed using the general linear model (GLM). The least significant difference (LSD) at 5% probability level was used in comparing the means.

RESULTS AND DISCUSSION

Seeds Germination

The extracts of millet residues were inhibitory to maize and millet seed germination than control (Figures 3-5). Pure millet extracts were more inhibitory than extracts of intercropped millet on maize but had little effect on seed germination of millet. Millet residues type (shoot, root, and biomass) had a similar impact, but all mixed residues inhibited maize and millet over control (Figure 4). The interaction of residues quantity and residues type inhibited the seed germination of maize. The pure millet shoots and root extracts were most inhibitory to seed germination of maize, while, intercropped millet root extract was least inhibitory (Figure 5). The interaction

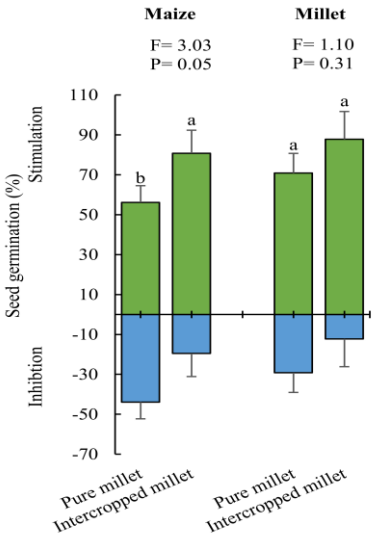

Figure 3. The effects of millet residue quantity (pure millet and intercropped millet) on seed germination of maize and millet. the mean followed by the different latter is not significantly different p≤0.05.

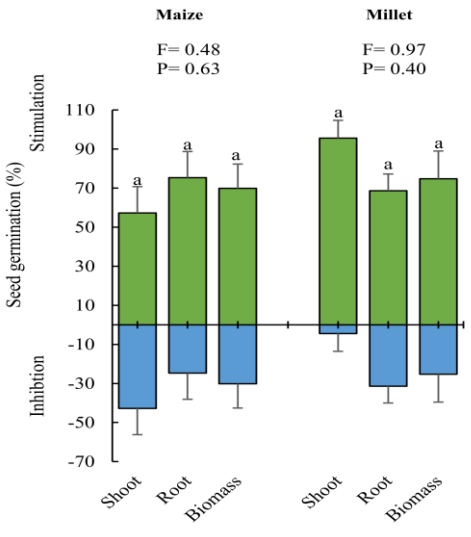

Figure 4. The effects of millet residue parts (shoot, root and biomass) on seed germination of maize and millet.

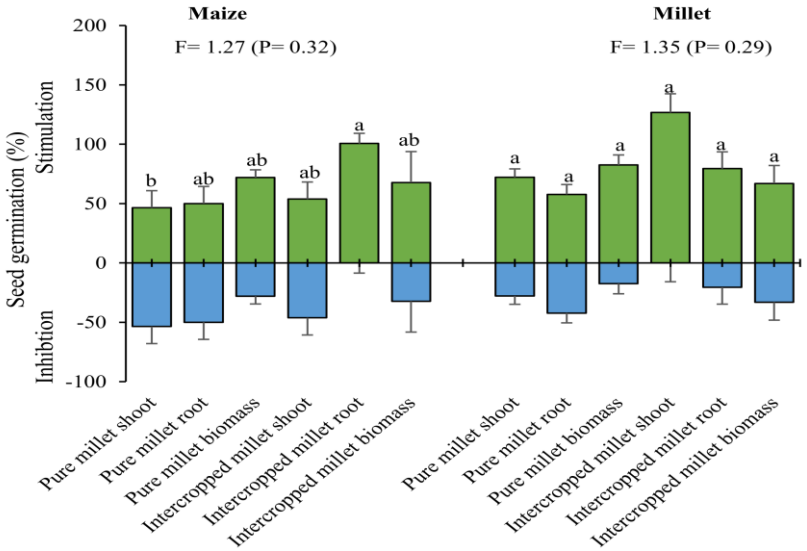

Figure 5. The effects of the interaction of residue quantity × residue type on seed germination of maize and millet.

of millet residue extracts concentrations and millet residues type did not affect the millet seed germination. However, the pure millet interaction was most inhibitory to millet, while the intercropped millet shoot had rather stimulatory effects.

The magnitude of inhibition intensified by increasing extracts concentrations i.e. was concentration dependent. Allelochemicals may be inhibitory to seed germination, growth and development of plants. Higher concentrations inhibits the seeds germination, probably due to high contents of allelochemicals, which may also cause complete inhibition (30). Similar inhibitory effects were caused by aqueous leaf extracts of proso millet on wheat (15).

The shoot and root millet residues extracts had allelopathic effects on the maize and millet seed germination and seedling growth. The pure millet residue extracts had negative effects and pure millet biomass (shoot+ root) was more inhibitory; this corroborates with some reports that found millet residues significantly inhibit some crops' germination (14,17,37). Higher concentrations of pure millet shoot and pure millet biomass extracts inhibited the seed germination through their allelochemicals contents (7,19). Furthermore, phytotoxicity might have been enhanced by the synergistic impacts of high concentrations of different phenolic compounds present in the extract (29). This study results are consistent with the determined allelochemicals in some past studies (3,36). The maize seed were most sensitive to millet residues extracts. The millet residues stimulated the millet seed germination, contradicting previous reports about smaller seeds being more sensitive to the allelochemicals present in millet residues (47). The allelopathic effects of millet residues extracts on seedlings growth of different target plants followed trends similar to seed germination.

The pure millet residues extracts were more inhibitory (43.87%) to maize germination than intercropped (19.54 %) and control (Figure 3). Pure millet extracts and their interactions were more inhibitory to maize germination than intercropped millet extracts. Target species varied in their sensitivity to millet residue extract concentrations, residue type extract and interaction. Maize proved most sensitive, because its germination was suppressed by millet residues extracts concentrations and interaction of residues extracts concentrations and residues type. Other studies support this sensitivity of subsequent crops to aqueous extracts of millet residues. *Tithonia diversfolia* and field bindweed extracts inhibited the germination of maize (34). Pearl millet and bindweed aqueous extracts inhibited the finger millet malt amylases by millet phenolics, and field bindweed extract inhibited the germination of millet (10,16, 39).

Germination Time

Mean germination time is an accurate measure of the time taken for seeds to germinate; instead, it focusses on the day when most germination events occurred. The lower mean germination time indicates faster seed germination. The mean germination time of maize was 3.14 days and in Proso millet was 1.56 days. There was no significant difference among the pure millet and intercropped treatments in maize (Figure 6). In comparison, the millet seed germination was delayed by pure millet followed by intercropped millet residues extracts and their types over control (Figure 6). There were no significant effects of millet residues type on seed germination time

of maize and millet. A gradual increase in germination time was recorded in maize seeds in

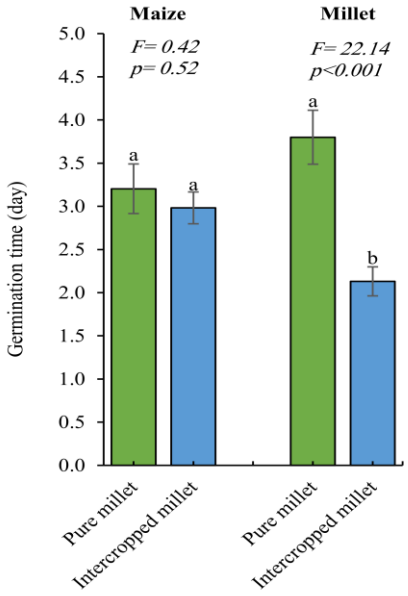

Figure 6. The effects of millet residue quantity (pure millet and intercropped millet) on the germination time of maize and millet.

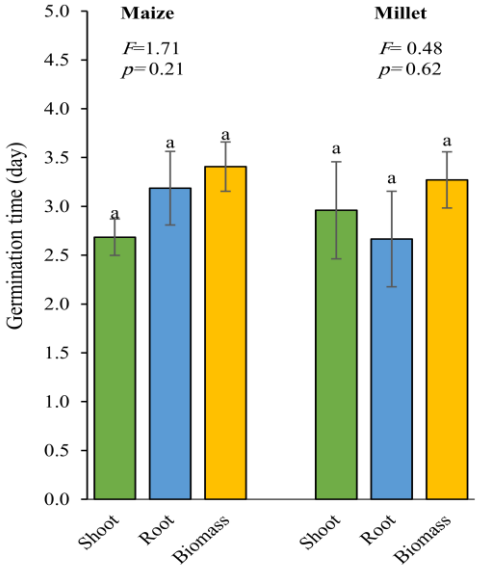

Figure 7. The effects of millet residue type (shoot, root and biomass) on the germination time of maize and millet.

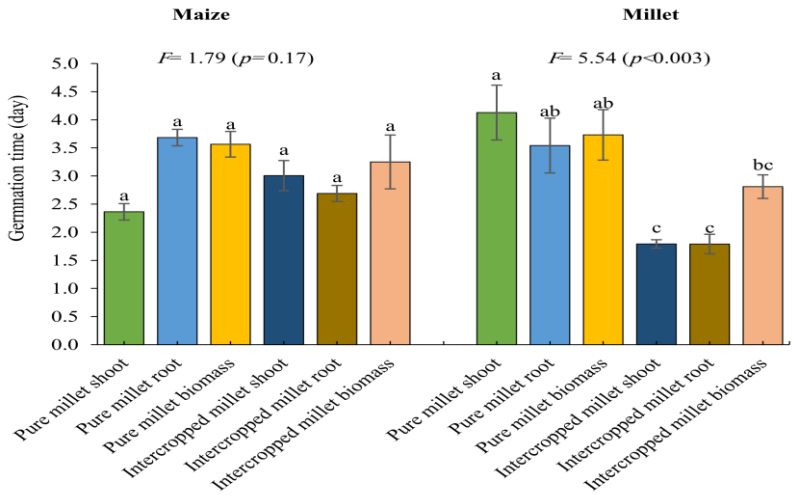

Figure 8. The effects of the interaction of residue quantity × residue type on the germination time of maize and millet.

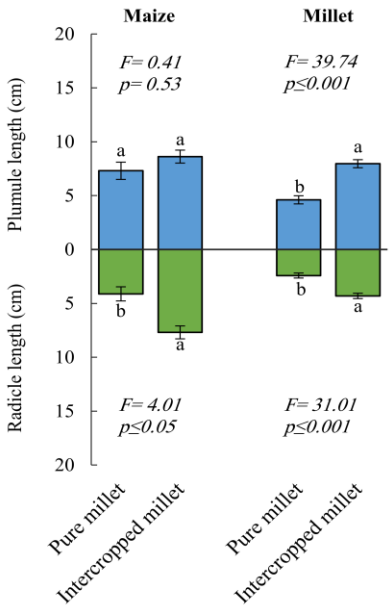

Figure 9. The effect of millet residues quantity (pure millet and intercropped millet) on the radicle length and plumule length of maize and proso millet.

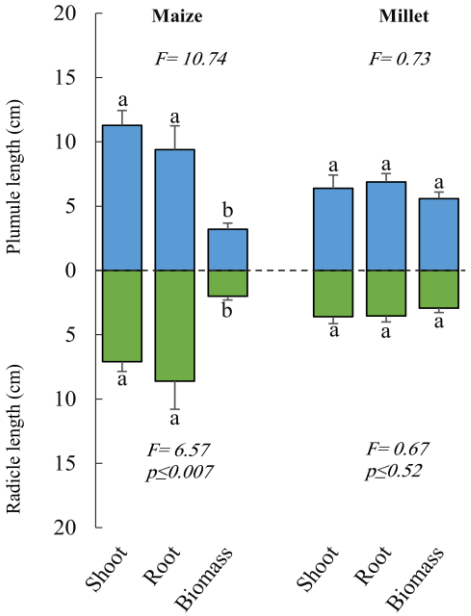

Figure 10. The effect of millet residues type (shoot, root and biomass) on the radicle length and plumule length of maize and proso millet.

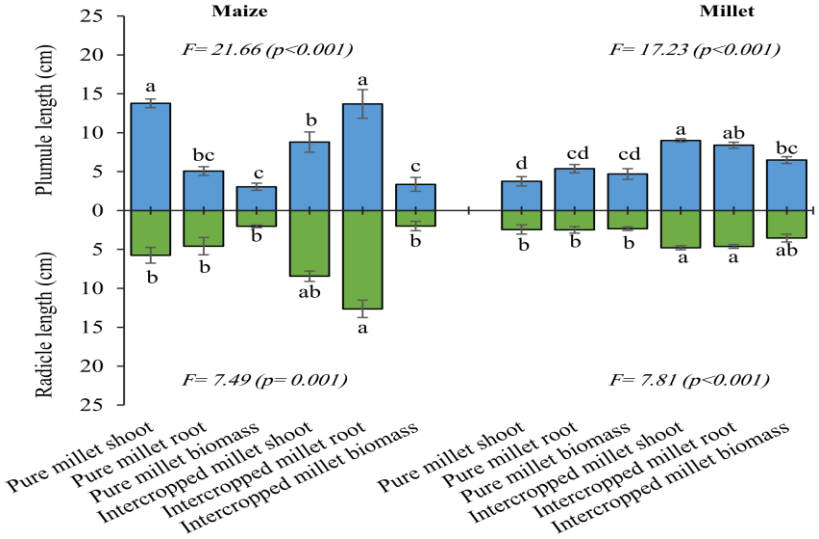

Figure 11. The combination effect of millet residues quantity and millet litter types on maize and millet radicle and plumule length.

response to shoot, root and biomass extract. However, the millet seeds showed variable responsess, with the shortest germination time under root extract (Figure 7).

There was significant difference among the different concentrations and different millet residues types (Figure 8). In millet, the interaction of pure millet shoot extracts delayed the seed germination than intercropped millet shoot, root, and biomass interaction. There was no significant effect of millet residue quantity and residue type interaction on maize germination time (Figure 8). There was significant difference in maize's mean germination time in different treatments (Figures 7 and 8), but had variable mean germination time. This implies that seeds can germinate across different time spread and but have the same mean germination time.

Seedlings growth

The radicle and plumule length of tested plants (Figures 9-11) were significantly influenced by millet residues type on the radicle and plumule length. In control, the radicle length was (14.22 cm) in maize and (6,59 cm) in millet, while the plumule was (12.53 cm) in maize and (10.44 cm) in millet, respectively. The millet roots extracts resulted in longest radicle length of maize seedlings, while it was shortest with biomass extract. While the plumule length was highest in shoot residues extracts, followed by root extracts and was the lowest in biomass extract. But the millet residues extracts did not affect the radicle and plumule length of millet seedlings. The millet residues extract type inhibited the radicle and plumule length of maize and millet seedlings. Both Pure millet and intercropped millet residues extracts exhibited inhibitory effects in test crops. Generally, radicle are more sensitive to residues extract than plumule (Figure 10). The millet residues concentrations inhibited the radicle length and plumule length than control (Figure 11). The intercropped and the pure millet extracts inhibited the radicle and plumule length of maize and millet seedlings. All millet extracts reduced the plumule length (Figure 11). The smallest radicle and plumule length were recorded in response to extracts of pure millet biomass and intercropped millet biomass on maize, while, in the intercropped millet root the length was highest. In millet seed germination, the radicle and plumule length responses were higher in the interaction of intercropped millet with root, while in shoot and pure millet intercropped with millet residues type was the lowest. The pure millet residues extract and millet shoots residues type extracts are more allelopathic than intercropped millet residues or millet’s root residues extract. Radicle growth was more sensitive to phytotoxic compounds in millet residues than plumule growth. The millet residue extracts concentration was inhibitory to radicle and plumule growth of maize and millet.

CONCLUSIONS

The extracts from shoots and roots of Proso millet proved inhibitory to test plants of maize and millet. All treatments inhibited the seed germination and seedling growth (radicle length, plumule length) of test crops over control crop. Thus, sowing of maize and millet after preceding crop of Proso millet should be avoided. Further research is required to identify the allelochemical components and their amount in Proso millet causing the inhibitory effects.

ACKNOWLEDGMENTS

This work was financially by the Strategic Priority Research Program of Chinese Academy of Sciences (Grant No. XDA20100102), Major Special Science and Technology Project of Gansu Province (Grant No. 18ZD2FA009), the Lanzhou City's Scientific Research Funding Subsidy to Lanzhou University and Innovative Research team of Chinese Ministry of Education (Grant No. IRT-17R50).

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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