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Allelopathic effects of proso millet (*Panicum miliaceum* L) extracts on seed germination and seedling growth of alfalfa and vetch

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ABSTRACT

In greenhouse experiment, 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 Common vetch (*Vicia sativa* L. var. Lanjian No.3) and Alfalfa (*Medicago sativa* var. WL168HQ). The millet residues extracts decreased the seed germination and seedlings growth (radical length, and plumule length) of test crops over control. All test crops showed similar responses of seed germination and seedlings growth in interactions of different types and different concentrations of all residue's extracts. The shoot, root, and biomass residue extract of proso millet inhibited the seed germination and seedlings growth of the recipient crops. Therefore, sowing of common crops like vetch and alfalfa on fields after previous proso millet should be avoided.

Keywords: Alfalfa, Allelopathy, inhibition, intercropping, *Medicago sativa, Panicum miliaceum,* Proso millet, residues, seed germination, seedling growth, vetch, *Vicia sativa.*

INTRODUCTION

Allelopathy is an interference mechanism, where plants released chemicals that affects the other plant's growth, population and communities (8,22). The allelopathic potential of plants depends on the biomass, plant density, solubility and adsorption of allelochemical in soil (34). The living and dead tissues (leaves, roots, stems, flowers) of plants during their decomposition release allelochemicals which causes allelopathic effects (6,16), suppress the growth of neighboring plants (11,37). The decomposition of plant residues increases the soil microbial activity, if legume crops residues are decomposed, they release nitrogenous compounds which promotes the growth of next crop (19,34). Many crops and their residues affect the seed germination and seedlings growth of other crops by releasing allelochemicals (12,14,23,25,30,33). The allelopathic interactions also occur between the crops in crop rotations (5).

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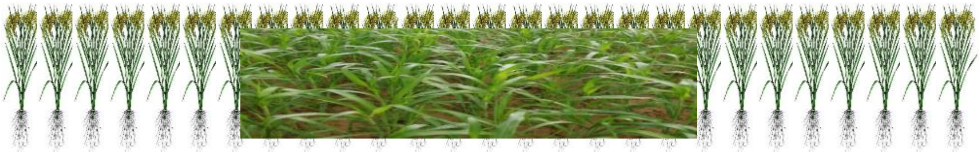

Pure proso millet (200000 plant ha^{-1})

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.

Common Vetch *(Vicia sativa L)*

Alfalfa (*Medicago sativa)*

Figure 2. The recipient plant seeds (common vetch, and alfalfa).

Proso millet (*Panicum miliaceum* L.) ranks sixth among the world's important cereals (7). Because its seeds are nutritious, hence, used as food and animal feed in China, Japan, India, and African countries, especially in the arid regions (2,7,30,24). About 20-varieties of proso millet are grown worldwide (17,36). Application of proso millet residues in wheat crop fields controls annual grass weeds, insect pests and diseases (10, 30). In winter crops, sown after millets, the weeds seed bank is reduced up to 90 % (3,4,15,28). The Proso millet yield decreased by 9.6 % in monoculture or no till conditions than when grown in crop rotation with bean (20). 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 (1,31). The stubble extracts of proso millet inhibits the seed germination and seedling growth of wheat (13,27).

The proso millet allelopathic potential is known (18,38). However, the effects of it’s 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 *Vicia sativa* L. and *Medicago sativa*.

MATERIALS AND METHODS

The study was conducted in a greenhouse at Yuzhong Agricultural Station, Lanzhou University, Yuzhong, Gansu Provence, China. [35.94°N, 104.15°E, Elevation : 1875 m]. The area has 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 shade at 30 ºC for ten days. The dry materials were grounded and passed through sieve (1 mm size). To prepare 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 powder 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 experimental 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; Distilled water was used as control. The extract was stored as stock solution in refrigerator at 4 ºC.

Petriplate bioassay

The experimental treatments consisted of 3 factors: (i). Proso millets 2 (Pure, intercropped) (Figure1), (ii). Proso millet parts 3 (shoot, root, biomass), (iii). Recipient crops 2 [vetch (*vicia sativa* L.) and Alfalfa (*Medicago sativa*)] (Figure 2). The treatments were replicated 10 times in Complete Randomised Design. Ten seeds of each recipient specie were sown in each sterilized Petri dish (9 cm dia) lined with two Whatman No.3 filter papers. Ten ml millet extracts of different concentrations were added to each Petri dish at the beginning. To keep Petri dishes moist, 5 ml extracts was added per petri dish on alternate days. The Petri dishes were kept in incubator at 25 ºC in dark. Seeds were considered germinated when 1 mm radical was visible. The numbers of seeds germinated were counted daily till 15 days. The following seeds germination parameters were determined

Seed's germination: The seed germination was calculated asper (21,37) as under:

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

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.

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 (26).

Radical and plumule length

A Measuring Scale was used to measure the radical and plumule length on 15th day after the germination. The inhibitory/Stimulatory (%) effects of extracts on seeds germination and seedlings growth of recipient plants were calculated as per Zhang and Fu (37). The results of extract-treated seeds were statistically compared with 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 millet residues extracts inhibited the seed germination of vetch and alfalfa species (Figures 3-5). Pure millet residues extracts were more inhibitory than intercropped millet residues on vetch, while the effect of millet residues quantity on alfalfa was not significant (Figure 3). Millet residues type viz., shoot, root and biomass significantly inhibited the seed germination of vetch and alfalfa than control (Figure 4). A variation in the effects of residue types was recorded. In case of vetch, the millet root residues were more toxic than shoot, but in alfalfa, the shoot residue was more inhibitory than millet roots residue. The interactions of residues quantity and residues type proved inhibitory to vetch (Figure 5). However, the interaction of millet residues quantity and millet residues type did not affect the seed germination of alfalfa. In vetch the pure millet roots extract was more inhibitory than the roots of intercropped millet. On the contrary, in alfalfa, intercropped millet biomass extracts were more inhibitory than pure millet biomass.

The inhibition increased with increase in various aqueous extracts concentrations i.e., was concentration dependent. Allelochemicals affects the seed germination, growth and development of plants. The higher concentrations adversely affect the seed germination and may also cause completely inhibition (25). Likewise, the aqueous leaf extracts of proso millet affects the seed germination of wheat (13).

The millet shoots and root residues extract had allelopathic effects on seed germination and seedling growth of common vetch and alfalfa. This corroborates with some reports that millet residues significantly inhibits the germination of some crops (12,14,29). The higher concentrations of millet inhibited the seed germination due to the presence of inhibitory allelochemicals (6,16). Furthermore, phytotoxicity may be enhanced by the synergistic impacts of different phenolic compounds present in high

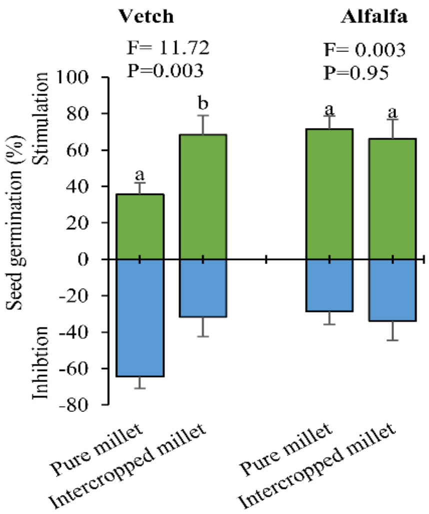

Figure 3. The effects of millet residue quantity on seed germination of vetch and alfalfa, the mean followed by the different latter is not significantly different p⩽0.05.

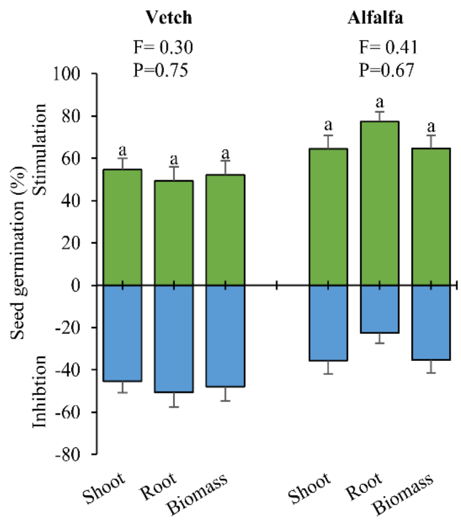

Figure 4. The effects of millet residue type on seed germination of vetch and alfalfa.

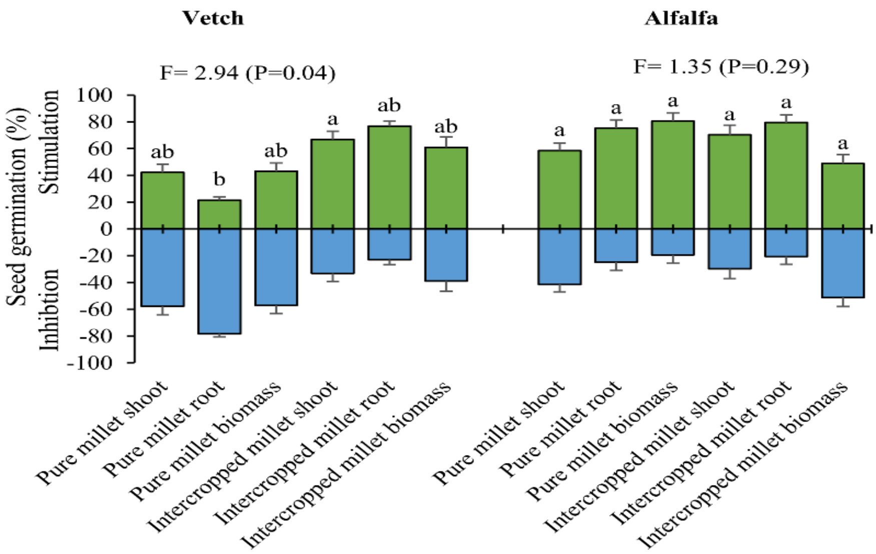

Figure 5. The effects of interactions of residue quantity × residue type on seed germination of vetch and alfalfa.

concentrations (25). This study results showed that the common vetch was most sensitive to millet residues extracts are consistent with allelochemicals determined in past studies (3,28). The allelopathic effects of millet residues extracts on seedling growth of vetch and alfalfa followed trends similar to seed germination.

Common vetch germination was inhibited by pure millet residues extracts up to 64.35 % of control and by intercropped up to 32.67 % (Figure 3). Target species varied in their sensitivity to millet residue extract concentration and type of residue. The vetch seed germination was most sensitive than alfalfa. Other studies support this sensitivity of subsequent crops species to aqueous extracts of millet residues. The aqueous extract from roots of Johnson grass had allelopathic effects on seed germination and initial development of soybean, pea and vetch (32). The aqueous extracts of black mustard causes inhibitory effects on germination and growth of alfalfa (35). The allelopathic influence of nine forage grasses extracts were also observed on germination and seedling growth of alfalfa (9)

Germination Time

Mean germination time is an accurate measure of the time taken for seeds to germinate; it also shows the day, when most germination events occurred. The lower germination time shows the faster seed germination rate. The seed germination of vetch was fastest with pure millet residues extract and was delayed by intercropped millet residues over control (Figure 6). On the contrary in alfalfa, seed germination was faster with intercropped millet residues extract than with pure millet extracts (Figure 6).

These variations in the germination time in response to the treatments of millet residues type on vetch and alfalfa were not significant (Figure 7). Minimum germination time was observed in both vetch and alfalfa in response to shoot residue extract, while, maximum time was taken under biomass treatment. A gradual increase in germination time was recorded with maximum delay under biomass treatment. However, in case of both vetch and alfalfa the difference in germination time in response to root and biomass was not significant.

The interactions of millet residues quantity and millet residues type are shown in Figure 8. There were significant differences among the various concentrations and millet residues types interactions. In vetch, the interaction of intercropped millet residues type with root and biomass delayed the germination, while the germination was faster in pure millet interaction with root. While in alfalfa, the interaction of pure millet shoot germination took longer than that of intercropped millet shoot, root, and biomass interaction (Figure 8). As shown in (Figure 7 and 8), there were significant differences in the mean germination time of different target plants with various treatments.

Seedlings growth

The radicle and plumule length of Alfalfa and vetch were shown in Figure 9-11. The millet residues type significantly influenced the radicle and plumule length of test crops. The highest radicle and plumule length were observed in the seedlings treated with root extracts. Whereas, the lowest radicle and plumule length was recorded in

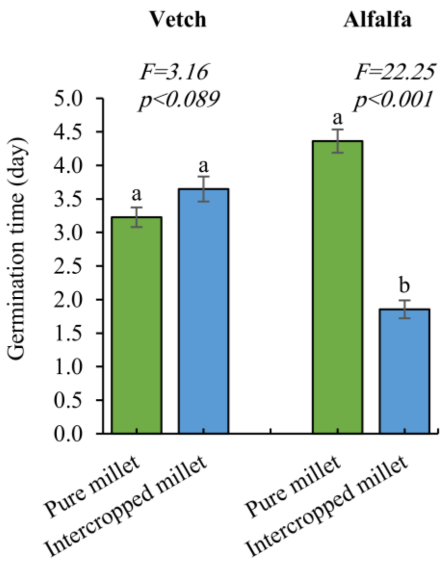

Figure 6. The effects of millet residue quantity on the germination time of vetch and alfalfa.

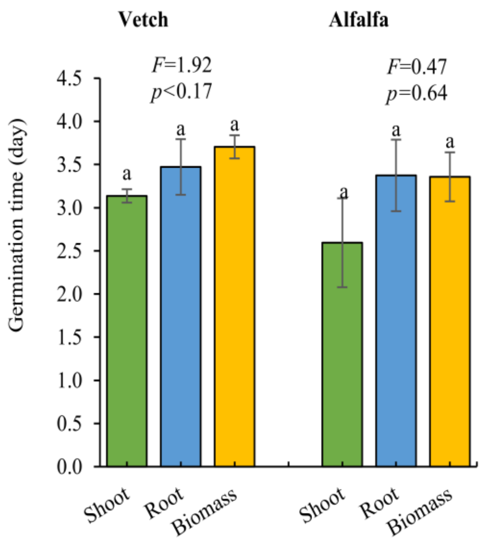

Figure 7. The effects of millet residue type on the Germination time of vetch and alfalfa

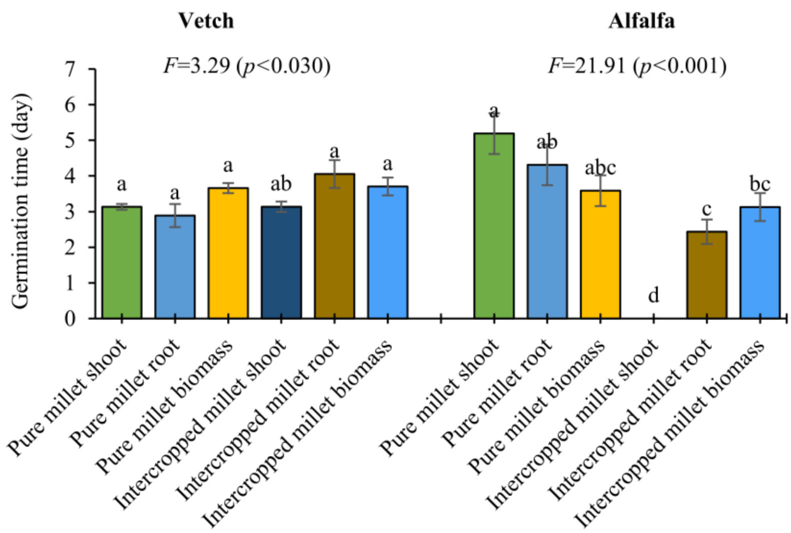

Figure 8. The effects of the interactions of residue quantity × residue type on the germination time of vetch and alfalfa.

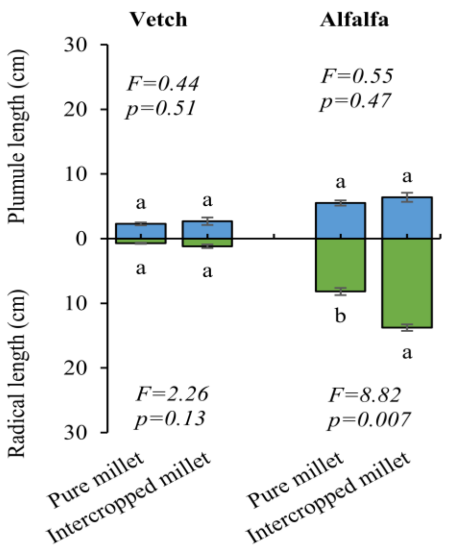

Figure 9. The effects of millet residues quantity on the radical length and plumule length of vetch and alfalfa.

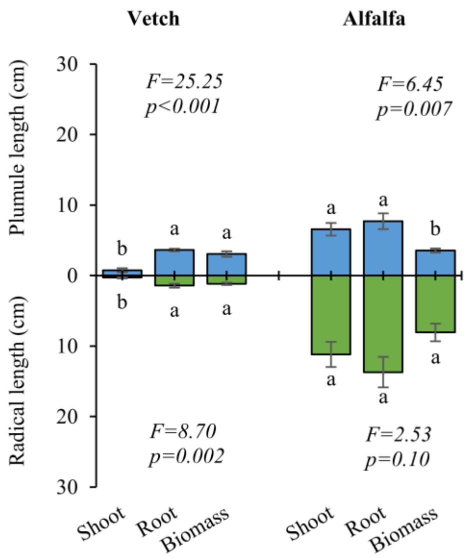

Figure 10. The effects of millet residues type on the radical length and plumule length of vetch and alfalfa.

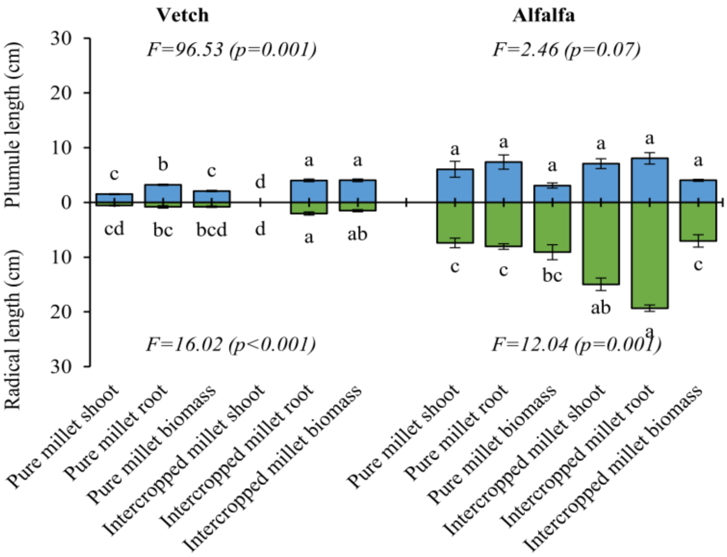

Figure 11. The combination effects of millet residues quantity and millet litter types on vetch and alfalfa radical and plumule length.

common vetch seedlings treated with shoot extract. The biomass extract was most inhibitory to seedlings growth of alfalfa.

The millet residues extract inhibited the radical and plumule length of common vetch and alfalfa seedlings than control. Generally, radicals are more sensitive to residues extract than the plumules (Figure 10).

The combination of millet residues quantity and extract type influenced the radicle and plumule length of tested plants. The intercropped millet root residues extract highly stimulated the radicle and plumule length of vetch and alfalfa (Figure 11), while the shortest radicle length of common vetch was recorded in pure millet shoot extracts. In alfalfa, the radicle length was maximum in intercropped millet root extract, while, the lowest radicle length was in the interaction of intercropped millet biomass extracts. In contrast, all millet extracts reduced the plumule length (Figure 11). In alfalfa, plumule length was longest in intercropped millet root extract and the smallest was recorded in extracts of pure millet biomass. The seedling growth of vetch and alfalfa showed that the millet shoots residues extracts were more allelopathic than that of millet's root residues extract. Residues extracts of millet inhibited the radicle elongation in vetch and alfalfa seedlings and the inhibition was more in vetch.

CONCLUSIONS

Allelopathy plays important role in farming systems. The shoots and roots extracts of proso millet proved inhibitory to seed germination and seedlings growth of test plants viz., Alfalfa and vetch. All treatments inhibited the seed germination and seedling growth (radical length, plumule length) of test crops over control. Thus, sowing of Common vetch and alfalfa after the preceding crops of proso millet should be avoided. Further research is required to identify the allelochemical components and their amount in proso millet residues causing the inhibitory effects.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest : The Authors declare no conflict of Interest.

REFERENCES

1. Altman, J. (2018). Pesticide interactions in crop production: *Beneficial and Deleterious Effects* **3**: 741-750.
2. Amadou, I., Gounga, M.E. and Le, G.W. (2013). Millets: nutritional composition, some health benefits, and processing-A review. *Emirates Journal of Food and Agriculture* **25**: 501-509.

3. Anderson, R.L., Bowman, R.A., Nielsen, D.C., Vigil, M.F., Aiken, R.M., and Benjamin, J.G. (1999). Alternative crop rotations for the central Great Plains. *Journal of Production Agriculture* **12**: 95-99.
4. Anderson, R.L. (2000). A cultural system approach can eliminate herbicide need in semiarid proso millet (*Panicum miliaceum*). *Weed Technology* **14**: 602-607.
5. Belz, R.G. (2007). Allelopathy in crop/weed interactions - An update. *Pest Management Science* **63**: 308-326.
6. Biosci, I.J., Hassannejad, S., Ghafarbi, S.P. and Lotfi, R. (2013). Allelopathic effects of wheat and barley on emergence and seedling growth of some weed species.*International Journal of Agriculture and Crop Sciences* **3**:128-134.
7. Changmei, S. and Dorothy, J. (2014). Millet-the frugal grain. *International Journal of Scientific Research and Reviews* **3**: 75-90.
8. Cheng, F. and Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science* **6**: 1-16.
9. Chung, Ill-Min and Miller, D.A. (1995) Allelopathic influence of nine forage grass extracts on germination and seedling growth of alfalfa. *Agronomy Journal* **87**: 767-772.
10. Das, S., Khound, R., Santra, M. and Santra, D.K. (2019). Beyond bird feed: Proso millet for human health and environment. *Agriculture* (Switzerland)**9**: 64-82.
11. Dhima, K.V, Vasilakoglou, I.B., Eleftherohorinos, I.G. and Lithourgidis, A.S. (2006). Allelopathic potential of winter cereals and their cover crop mulch effect on grass weed suppression and corn development. *Crop Science* **46**: 345-352.
12. Di Lonardo, D.P., Manrubia, M., De Boer, W., Zweers, H., Veen, G.F. and Van der Wal, A. (2018). Relationship between home-field advantage of litter decomposition and priming of soil organic matter. *Soil Biology and Biochemistry* **126**: 49-56.
13. Et, Q. (2014). Phytotoxic effects of millet seeds extract on germination and seedling growth of chick pea, red beans and mung beans in Pakistan. *Journal of Biology* **4**: 187-190.
14. Fischer, N.H., Weidenhamer, J.D. and Bradow, J.M. (1989). Inhibition and promotion of germination by several sesquiterpenes. *Journal of Chemical Ecology* **15**: 1785-1793.
15. Ghimire, R., Mesbah, A., Nilahyane, A., Ghimire, B. and Beck, L. (2019). Efficacy of cover crops on weed suppression, wheat yield and water conservation in winter wheat–sorghum–fallow. *Crop Science* **59**: 1745-1752.
16. Głąb, L., Sowiński, J., Bough, R., and Dayan, F.E. (2017). Allelopathic potential of sorghum *(Sorghum bicolor*(L.) Moench) in weed control: A comprehensive review. *Advances in Agronomy* **145**: 43-95.
17. Habiyaremye, C., Matanguihan, J.B., D'Alpoim Guedes, J., Ganjyal, G.M., Whiteman, M.R., Kidwell, K.K., and Murphy, K.M. (2017). Proso Millet (*Panicum miliaceum* L.) and its potential for cultivation in the pacific northwest, U.S.: A Review. *Frontiers in Plant Science* **7**: 1-17.
18. Han, G.J., Gou Y., Zhou Y. and Su P.K. (2018). Study on allelopathy of *Panicum Miliaceum* L. *Journal of Baicheng Normal University* **32**:3-6. (Chinese).
19. Jabran, K., Mahajan, G., Sardana, V. and Chauhan, B.S. (2015). Allelopathy for weed control in agricultural systems. *Crop Protection* **72**: 57-65.
20. Joshi, N.L., Dayal, D., Saxena, A., Kumawat, R.N., Singh, I., Bhati, D.S. and Singh, A.K. (2009). Agronomic management for sustainable crop production in arid environment. *Trends in Arid Zone Research in India* **I**: 278-353.
21. Kader, M.A., (2005). A comparison of seed germination calculation formula and the associated interpretation of resulting data. *Journal and Proceeding, the Royal Society of South Wales* **138**: 65-75.
22. Khanh, T.D., Xuan, T.D. and Chung, I.M. (2007). Rice allelopathy and the possibility for weed management. *Annals of Applied Biology* **151**: 325-339.
23. Khayamim, S., Afshari, R., Sadeghian, S.Y., Poustini, K., Roozbeh, F. and Abbasi, Z. (2014). Seed germination, plant establishment, and yield of sugar beet genotypes under salinity stress. *Journal of Agricultural Science and Technology* **16**: 779-790.
24. Kothari, S.L., Kumar, S., Vishnoi, R.K., Kothari, A. and Watanabe, K.N. (2005). Applications of biotechnology for improvement of millet crops: Review of progress and future prospects. *Plant Biotechnology* **22**: 81-88.
25. Mousavi, S.S., Karami, A., Haghighi, T.M., Alizadeh, S., Maggi, F. (2021). Phytotoxic potential and phenolic profile of extracts from *Scrophularia striata*. *Plants* **10**: 1-18.
26. Luo, Y., Zhao, X., Li, Y. and Wang, T. (2017). Effects of foliage litter of a pioneer shrub *(Artemisia halodendron)* on germination from the soil seedbank in a semi-arid sandy grassland in China. *Journal of Plant Research* **130**: 1013-1021.

27. Reigosa, M.J., Pedrol, N. and Gonzlez, L. (2002). *Allelopathy*. Science Publishers, Inc.USA. pp. 209-226.
28. Rice, J.R. (1992). Dislocation nucleation from a crack tip: An analysis based on the Peierls concept. *Journal of the Mechanics and Physics of Solids* **40**: 239-271.
29. Saeed, M.M., Ahmad, M., Ali, A. and Cheema, M.N. (1992). Effects of a tertiary amine on the synergic extraction of lanthanides by Thenoyltrifluoroacetone (HTTA). *Radiochimica Acta* **57**: 125-130.
30. Santra, D.K. (2013). *Proso Millet Varieties for Western Nebraska.* University of Nebraska-Lincoln NebGuide **G, 2219.**
31. Saxena, A., Singh, D.V, and Joshi, N.L. (1996). Autotoxic effects of pearl millet aqueous extracts on seed germination and seedling growth. *Journal of Arid Environments* **33**: 255-260.
32. Shteliyana, K., Irena, G., Angel, H. and Anna, I. (2012). Allelopathic effects of aqueous extract from root systems of Johnson grass on seed germination and initial development of soybean, pea and vetch. *Ratar. i Povrt.* **49**: 250-256.
33. Sisodia, S. and Siddiqui, M.B. (2010). Allelopathic effects of aqueous extracts of different parts of *Croton bonplandianum* Baill. on some crop and weed plants. *Citeseer* **2**: 22-28.
34. Taylor, P. (2001). Selective isolation and screening of soil microorganisms for metabolites with herbicidal potential. *Journal of Crop Production* **4**: 197-218.
35. Turk, M.A., Shatnawi, M.K. and Tawaha, A.M. (2003). Inhibitory effects of aqueous extracts of black mustard on germination and growth of alfalfa. *Weed Biology and Management* **3**: 37-40.
36. Wen, Y., Liu, J., Meng, X., Zhang, D. and Zhao, G. (2014). Characterization of proso millet starches from different geographical origins of China. *Food Science and Biotechnology* **23**: 1371-1377.
37. Zhang, C. and Fu, S. (2010). Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops. *Allelopathy Journal* **26**: 91-99.
38. Zhang, L., Liu, R. and Niu, W. (2014). Phytochemical and antiproliferative activity of proso millet. *PLoS ONE* **9**: 64-82.

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