Journal of Bioresource Management

Volume 3 | Issue 1 Article 5

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Recommended Citation

Waris, A., Waris, L., Khan, M. A., & Shad, A. A. (2016). Allelopathic Effect of Methanol and Water Extracts of *Camellia sinensis* L. on Seed Germination and Growth of *Triticum aestivum* L. and *Zea mays* L., *Journal of Bioresource Management, 3* (1).
DOI: https://doi.org/10.35691/JBM.6102.0043
ISSN: 2309-3854 online

ALLELOPATHIC EFFECT OF METHANOL AND WATER EXTRACTS OF *Camellia sinensis* L. ON SEED GERMINATION AND GROWTH OF *Triticum aestivum* L. AND *Zea mays* L.

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ABSTRACT

This study describes the possible effects of tea residues on crop production. To investigate the negative effects of tea on two cereal crops, i.e. wheat and maize, laboratory experiments were conducted during May 2014 and repeated in June 2014 to study the effect of black tea extract on wheat and maize seeds' germination and growth. The experiment was conducted in petri dishes and laid out in completely randomized designs, replicated thrice. The petri dishes were kept in a growth chamber, with the temperature set at 25°C. 50 g of each dried fresh and used black tea was separately soaked in 500 ml of hot and cold distilled water. The same amount of tea residue was soaked in 500 ml of methanol. Ten seeds of each wheat variety (Siran) and maize variety (Azam) were placed in each petri dish. 10 ml of each extract was applied to each petri dish according to the requirement. A control (distilled water) was used for comparison. Analysis of the data revealed that tea extract significantly suppressed seed germination and the growth of wheat and maize. Methanol extracts, on the other hand, completely inhibited seed germination. The negative effects of tea extracts on seed germination of crops warns that apart from polluting the soil, the crop production could be greatly affected by dumping tea waste in agricultural fields. However, used cautiously, the application of tea extract can be used to suppress the growth of weeds in agriculture.

Keywords: Allelopathy, tea, wheat and maize, tea waste.

Introduction

Black tea is the most popular beverage in Pakistan, and all over the world, and is the second most important drink after water. Tea is prepared from the leaves of the tea plant (*Camellia sinensis* L. Kuntz), belonging to the family Theaceae and is used as a beverage at home and elevated social events. Various kinds of tea from this plant have been prepared for thousands of years. There are three primary categories of tea from *Camellia senensis* based upon three different states of oxidation of the leaves: green, oolong and black tea. Green tea is made from leaves that have undergone only a slight degree of oxidation. Oolong tea has been subjected to more oxidation, while black tea has been extensively oxidized. China is the birthplace of tea, followed by India, Kenya, Turkey, Indonesia, Bangladesh, Sri Lanka and Japan. Tea is used in Chinese and Indian traditional medicine. Clinical studies have documented the human health benefits of tea, especially for its role as a cancer preventing anti-oxidant (IFI CLAIMS Patent Services ©2012 Google). As per the latest Food and Agriculture Organization (FAO) data, world consumption of tea rose by 4 percent to 4 million tons in 2010 (Anonymous, 2010).

Pakistan is one of the world's largest importers and consumers of tea as over 120,000 tons of tea are imported each year. Pakistan has a market of over 140 to 170 million kg for black tea (http://www.pakistantoday.com.pk). However, there is no utilization of the used tea in Pakistan and it is thus wasted. This waste pollutes the environment in many ways as a large quantity of tea residue is thrown away. There is a possibility of exploiting the used tea for consumption as a fertilizer and/or herbicide for weed control. There is a colossal demand for organic farming all over the world. The global market for organic foods is more than $23 billion per annum and is growing rapidly (Roseboro, 2006). In organic farming, the use of crop residue is an excellent choice to improve soil health and make the nutrient deficient soil fertile. This approach will also improve the soil chemistry.

In the agricultural field, like other crops, tea (*Camellia sinensis)* and its residues have been reported to have an allelopathic potential to suppress weeds or enhance crop production as there are certain chemicals that are secondary metabolites, synthesized by tea plant (Rezaeinodehi *et al*., 2013; Dibah *et al.,* 2012). Response of these chemicals is concentration dependent (Einhellig, 1986) ; delaying the plant growth at high concentrations and enhancing at low concentrations (Subtain *et al.,* 2014). Allelochemicals reported in tea (*Camellia sinensis* L.) are alkaloids and phenolic compounds (Adnan *et al.,* 2013). Inhibitory effect of tea organs (leaf, flower and fruit) at different concentrations on germination and growth of garden cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), redroot pigweed (*Amaranthus retroflexus* L.) and golden foxtail (*Setaria glauca* L.) are already reported (Rezaeinodehi *et al*., 2013). They further reported that tea extract stimulated germination of these plants at low concentrations and inhibited as concentration was increased. They suggested that tea, as an allelopathic plant, could be used as natural herbicide or growth promoter in weed control and crop management programs. Dibah *et al.* (2012) also reported allelopathic potential of tea leaves extracts of different developmental stages on *Vicia* spp.

Previous research has suggested tea wastes as soil conditioner to improve soil chemical properties and plant growth (Abdulghani, 2012)**,** metal adsorbents (Azmat *et al.,* 2006), and compost (Mastouri, 2005). Tea leaf wastes were recycled to improve the yield and mineral content of grains of paddy rice (Morikawa and Saigusa, 2008). However, this use is neglected in developing countries and thus is applied to agricultural lands as fertilizer and/or landfill, though tea residues offer unlimited opportunities to be used in agriculture. Such approaches will enable switching towards ways which are organic, eco-friendly and sustainable. Tea residue is a low cost and more than 80 million tons produced per year, it could be a crucial option for the green industry.

Keeping in view the importance of increasing use of black tea and the consequent increase in the used tea residues, lab based studies were initiated to investigate the effectiveness of extracts of black tea for possible effects on seed germination and growth of wheat and maize. Thus any possible impact on these two major crops could be further elaborated for their use in agriculture.

Materials and methods

Two laboratory based experiments were conducted to investigate the effect of tea extracts on the germination and growth of wheat and maize at Weed Science Research laboratory, Department of Weed Science, The University of Agriculture, Peshawar, Pakistan during May, 2014. In June 2014, the experiment was repeated in the same conditions. The experiments were laid out in Completely

Randomized Design (CRD) and replicated thrice. The seeds of wheat cultivar “Siran” and maize cultivar “Azam” are widely grown in the area and thus selected for studies. Fresh black tea was purchased from local market and the used tea residue was obtained from the cafeteria of The University of Agriculture Peshawar, Pakistan.

Extraction and purification

Used tea residue was fully dried in sunlight. After drying 50 g of each fresh and used tea was separately soaked in 500 ml of hot and cold distilled water. The same amount of tea was also soaked in 500 ml of methanol. All the samples were placed at room temperature for 24 hours. Each solution was filtered through four layers of cheese cloth to remove debris and finally through Whatman No.1 filter paper. Each stock solution was kept in the refrigerator till use.

Seed culture and treatments

Seeds of wheat were surface sterilized with water: bleach (10:1 v/v) solution for 10 minutes to avoid contamination and were thoroughly rinsed several times with sterile water. For testing, 21 Petri dishes of 9 cm were washed, dried and then sterilized in autoclave at 110 -120°C for 1 hour. Whatman No.1 filter papers were kept in each Petri dish and ten seeds of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were placed in separate Petri dishes at equal distances. All the experimental Petri dishes were kept at room temperature of 23°C for 10 days. 10 ml of each extract was added to the petri dishes and each treatment was repeated three times. Seeds dsoaked in distilled water was used as control. 10 ml distilled water was applied to each petri dish during the experiment to keep the Watman paper moist for seedling development for a period of 10 days. The treatments were arranged in the following manner;

Treatments (extracts)

T1=Hot aqueous extract of fresh tea @ 1:10 (w/v)
T2=Hot aqueous extract of used tea @ 1:10 (w/v)
T3=Cold aqueous extract of fresh tea @ 1:10 (w/v)
T4=Cold aqueous extract of used tea @ 1:10 (w/v)
T5=Methanol extract of fresh tea @ 1:10 (w/v)
T6=Methanol extract of used tea @ 1:10 (w/v)
T7=Control (distilled water) @ 10 ml

During the course of the experiment, data were recorded on the variables mentioned below;

Germination Percentage (%)

Germinated seeds for each species (wheat and maize) were counted daily. The seeds were considered as germinated when the radical size was 2 mm. The number of germinated seeds was recorded after every 24 hour till 10 days after the start of the experiment. The percentage data were calculated using the formula,

$$\text{Germination Percentage} = \frac{\text{Germinated seed x 100}}{\text{Total seeds sown}}$$

Mean germination time (MGT)

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981).

$$MGT = \Sigma TiNi/S$$

Where, Ti=Number of days after beginning of the experiment
Ni=Number of seeds germinated on the day
S=Total number of seeds germinated.

Germination index (GI)

The germination index (GI) was calculated by suing the following formula.

$$GI = n/d$$

Where, n= number of seeds emerging on the day,
d=days after setting the seeds for germination

Seedling vigor index (SVI)

Seedling vigor index (SVI) was calculated according to the formula of Abdul-baki and Anderson (1973). SVI = Germination% × seedling length (cm)

Inhibition %

Inhibition **%** was recorded by comparing with control and using the formula of Hong *et al.* (2003). Inhibition% = [1-(sample extract/control)] x 100

Seedling length (cm)

Seedling length of maize and wheat was measured with a scale and average length was calculated in cm.

Fresh weight plant^{-1} (mg)

Fresh weight of seedlings was measured with the help of electronic balance and then average fresh biomass was calculated in mg.

Statistical analysis

The data recorded individually for each variable were analyzed statistically by using Analysis of Variance (ANOVA) technique and means (in case significantly different) were compared using least significant difference (LSD) test (Steel *et al*., 1997). The data presented in the figures are the average of two experiments.

RESULTS AND DISCUSSION

Germination (%)

Tea extracts significantly decreased seed germination of wheat($P \leq 0.01$) and maize ($P \leq 0.01$) (Fig.1). Wheat showed maximum germination (80%) in control (distilled water) with no germination in methanolic extract treatments. While the germination in all other treatments (tea extracts) depicted that seed germination of wheat was significantly decreased. Overall, the germination in all the treatments of tea extracts was statistically at par. However, the numerical values in cold water were lower than those recorded in hot water. The trend of germination for maize was similar to wheat. However, the germination percentage of maize was higher than wheat probably due to inherent characters. In methanolic extract treatments, no germination was observed for maize, as well as wheat.

The present studies showed that use of tea extracts negatively affected the seed germination of wheat and maize as compared to control. In developing countries, the tea residues are dumped in agricultural fields. Thus the seed germination of the following crop can be negatively affected due to tea residues. As addition of tea residue to soil is a continuous process independent of summer and winter therefore, the extraction of chemicals from tea residue to soil is expected. The addition of tea residue to soil is always on small scale due to limited quantity. However, continuous addition to soil may prove detrimental in a long run. On the other hand, there are possibilities of using tea residue for weed suppression in agricultural crops. However, the selective use of tea residues for weed suppression without affecting crop seeds will need more research. Using tea residues as band application in wide row crops, such as, vegetables and maize may prove effective. Nevertheless, field studies are required to validate these hypotheses. In a similar studies, Ahmed et al. (2015 and Sharma et al. (2014) claimed that extracts of plants has significant effects on the other plants. Use of fresh tea is uneconomical and practically impossible, especially on commercial scale. However, the use of used tea may prove feasible and economical for organic growers.

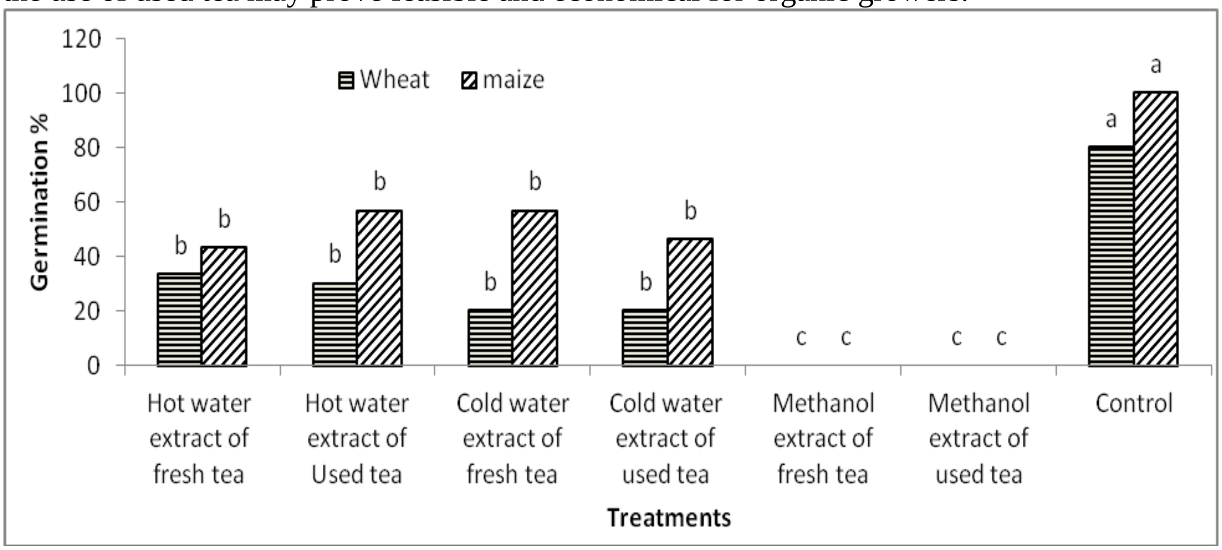

Fig.1. Effect of tea extracts on the germination% of wheat and maize seeds.

Mean germination time (MGT)

The rapidity of the germination was expressed by the value of mean germination time (MGT) i.e. the lower the value of the MGT the earlier the germination. Analysis of variance of the data revealed that different tea extracts had a significant effect on MGT of the wheat ($P \leq 0.01$) but had no effect on maize seeds (Fig. 2). MGT (4) was recorded for cold water extract of fresh tea that was statistically at par with hot water extract of fresh tea (3.5), control (3.42) and cold water extract of used tea (3.06). The lowest MGT (2.5) was observed for hot water extract of used tea. The data for maize (Fig.2) showed that MGT was not affected by the different tea extracts. Result showed that tea residues can enhance the speed of wheat seed germination . Thus tea residues can be used as a source of fertilizer in agriculture. In a similar study, Azmat *et al.* (2006) observed that tea waste increased the speed of germination of *Vigna rediata* and lettuce seeds (Mastouri, 2005). The germination speed of crop plants is important in crop-weed competition models. Therefore, the use of tea residues to suppress weed seed germination may provide benefits to the farmers. However, field application may also suppress weed seed germination. One option is to treat the field with tea residues well before sowing of crop. Thus weed seed may show poor germination. Tea residues are wasted in each home without any use therefore, the use of residues in this way might be economical in many developing countries. However, the practical application of tea residues needs more extensive studies at various doses against different crops.

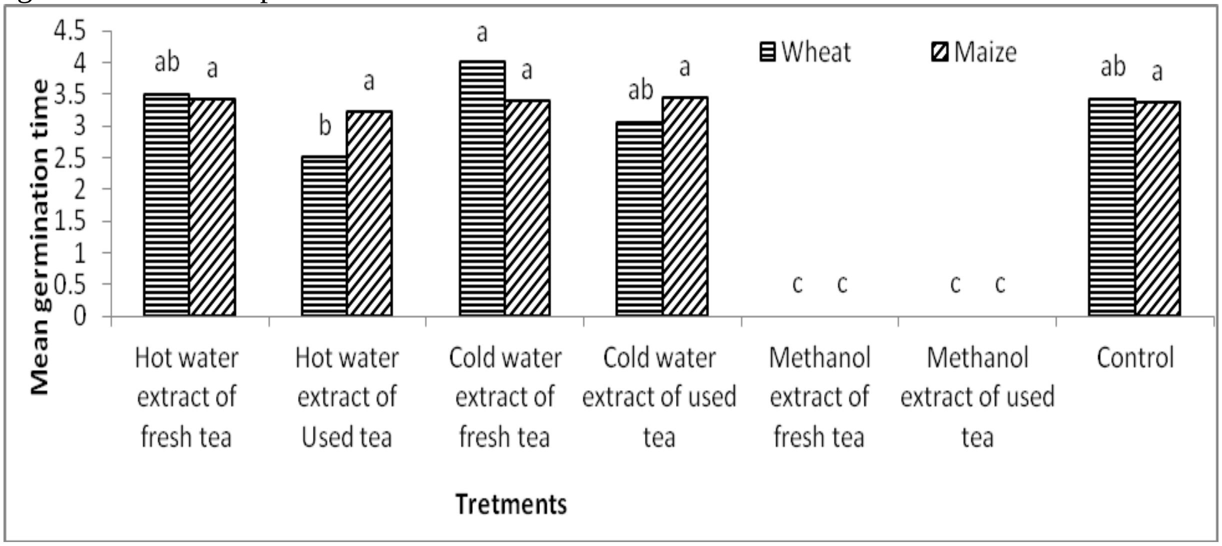

Fig. 2. Effect of tea residue extract on mean germination time of wheat and maize seeds.

Germination index

Germination index (GI) is directly correlated with germination percentage. Thus greater the value of GI, the greater will be germination percentage. The statistical analysis of data (Fig. 3) revealed significant differences in GI of wheat and maize due to different tea extracts. The data for wheat and maize showed that maximum GI was observed in control. All the tea extracts decreased the GI overall. However, the reduction of GI was dependent on the cold and hot water extracts in case of wheat, not maize. Wheat GI was higher in hot water extracts, irrespective of used and fresh tea. Thus it seems that hot water probably pulled out more chemicals from tea, which influenced the germination process. Consequently, this increased GI as compared to cold water. In light of these findings, it is concluded that tea residue extracts decrease germination index of both wheat and maize. While reporting the results of a study, Ahmed et al. (2015) observed that phytotoxicity of water extracts of *P. lagopus* resulted in total failure of germination of *B. pilosa* and *P. major*.

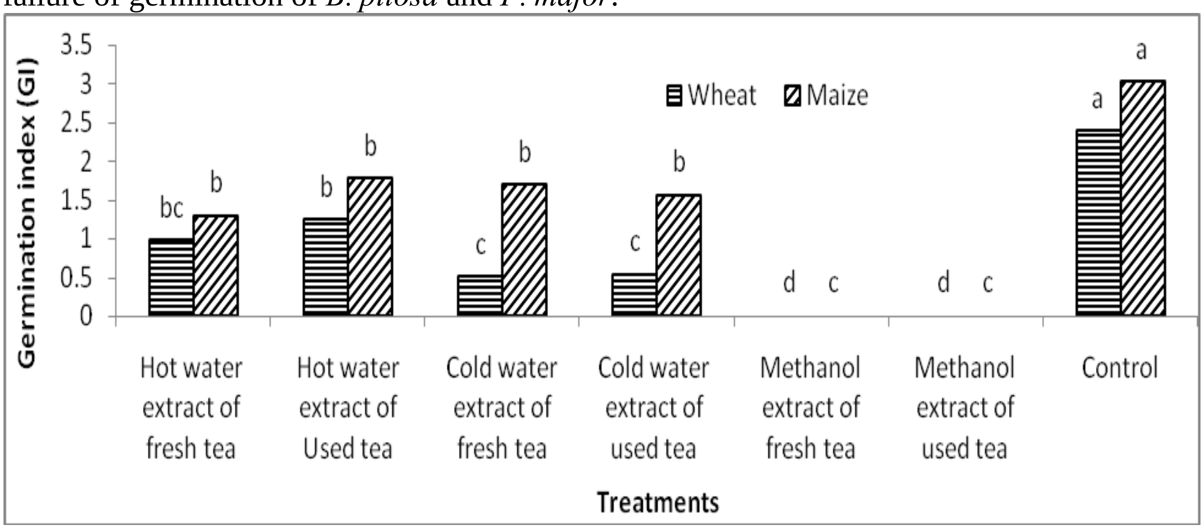

Fig.3. Effect of tea residue extract on Germination Index of wheat and maize seeds.

Seed Vigour Index

The level of activity and performance of seeds during germination and seedling emergence is expressed by seed vigor index (SVI). The data for wheat and maize (Fig. 4) showed that maximum value of seed vigour index was found in control treatment. While the SVI in all other treatments was substantially lower than control. SVI is an important parameter in plant science that determines plant germination and growth . The negative effect of extracts on SVI showed that tea extracts used in hot or cold water and taken from used or fresh tea are detrimental for plant growth. Thus dumping of tea waste without composting should be discouraged in agricultural fields to avoid crop losses. These results showed that addition of tea wastes can make the crop weaker. Moreover, the effect of tea extracts against soil micro flora should be investigated. Addition of tea residues or extracts might have positive or negative effects on soil micro-organism, whether beneficial or harmful. This will open new window to explore use of tea in agriculture. In analogous studies, Sharma *et al.* (2014) reported that seed vigour index can be improved by hydropriming.

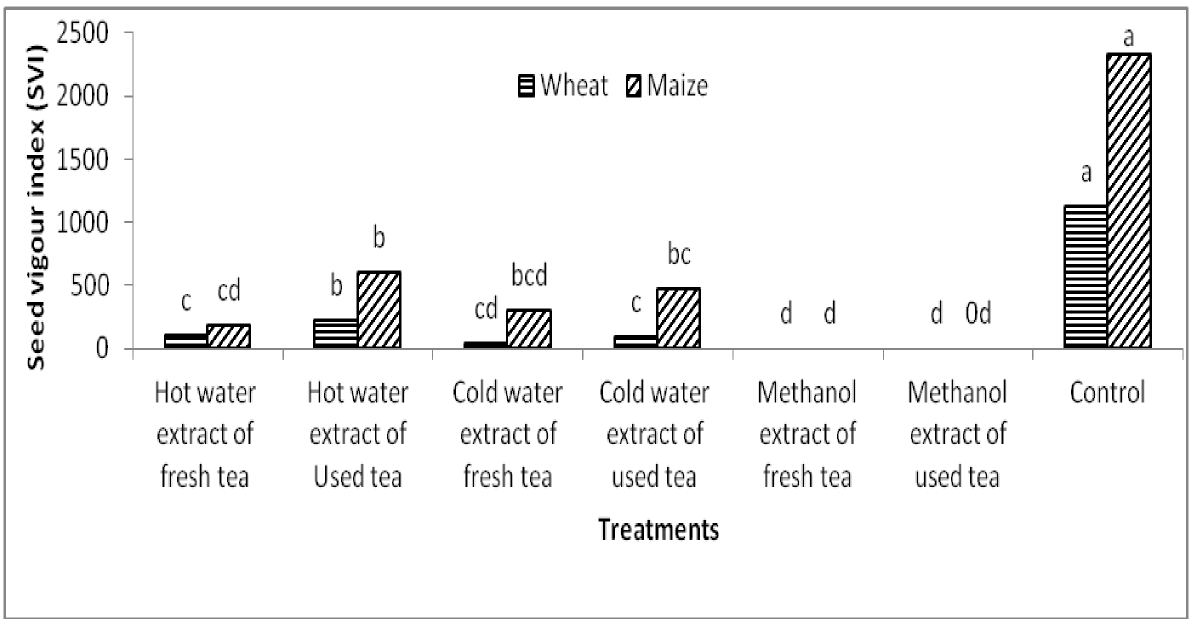

Fig. 4. Effect of tea residue extract on Seed Vigour Indices of wheat and maize seeds.

Inhibition %

Methanolic extracts of tea residues inhibited wheat and maize seed germination by 100 % (Fig. 5). Wheat and maize were equally affected by tea extracts. The inhibition of wheat and maize was independent of the effect of used or fresh tea. Similarly, hot water and cold water extracts, both gave statistically similar values of inhibition. This result concluded that application of tea residue extract may suppress seed germination which could be an option to use this method for weed control in agriculture by using proper concentration. However, using tea wastes in favour of crops and against weeds need more scientific studies and explanation. It is inferred that used tea improves the soil condition by adding organic matter, nutrients, and improves the pH of alkaline soils (Anonymous, 2015). This reveals that though tea may have an inhibitory effect on seed germination, in low concentrations it can be used for growing plants that are propagated vegetatively, especially horticultural crops.

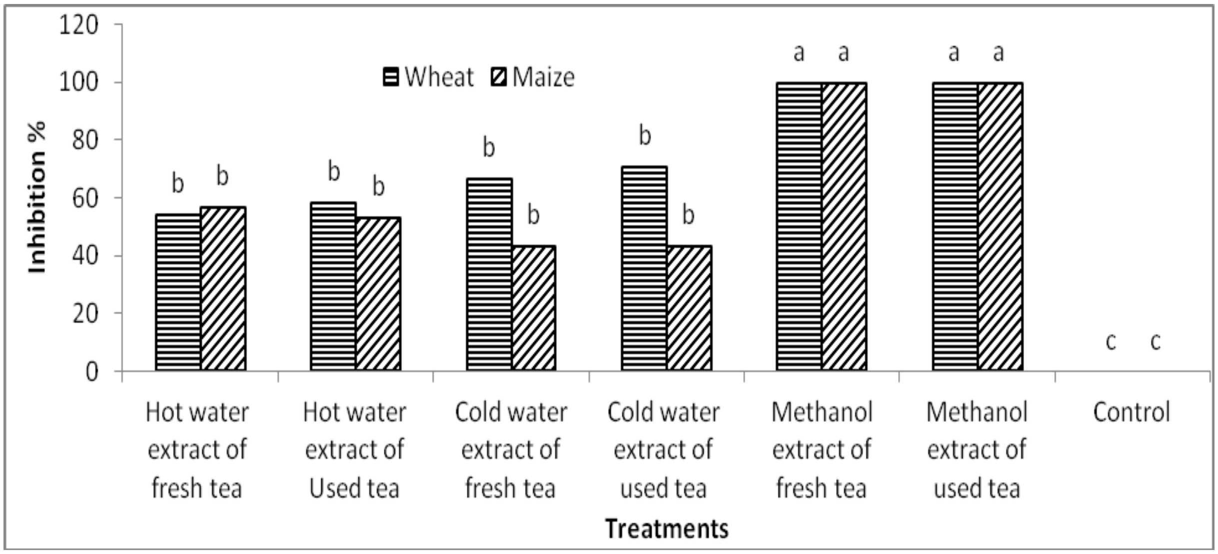

Fig. 5. Effect of tea residue extract on inhibition(%) of wheat and maize seed germination.

Seedling length (cm)

Statistical analysis of data showed that different tea extracts significantly affected the seedling length (cm) of wheat and maize (Fig. 6). Maize being inherently bigger and taller in size, showed more seedling length as compared to wheat. Used tea extract in hot water produced more seedling length in wheat compared to other treatments. Maize, on the other hand, produced longer seedlings in used tea extracts irrespective of cold or hot water in comparison with fresh tea extracts. The results followed a similar pattern as in seed vigour index (fig, 4 above). This is probably due to the vigour of the seedlings in their respective treatments that resulted in increased seedling length. Ahmed *et al.* (2015) claimed that the phytotoxicity of water extracts of *P. lagopus* resulted in total failure of germination of *B. pilosa* and *P. major*. They observed that higher concentrations were more harmful as compare to lower concentrations of extracts. In addition, the radicle and plumule were also inhibited by the water extracts of the plant.

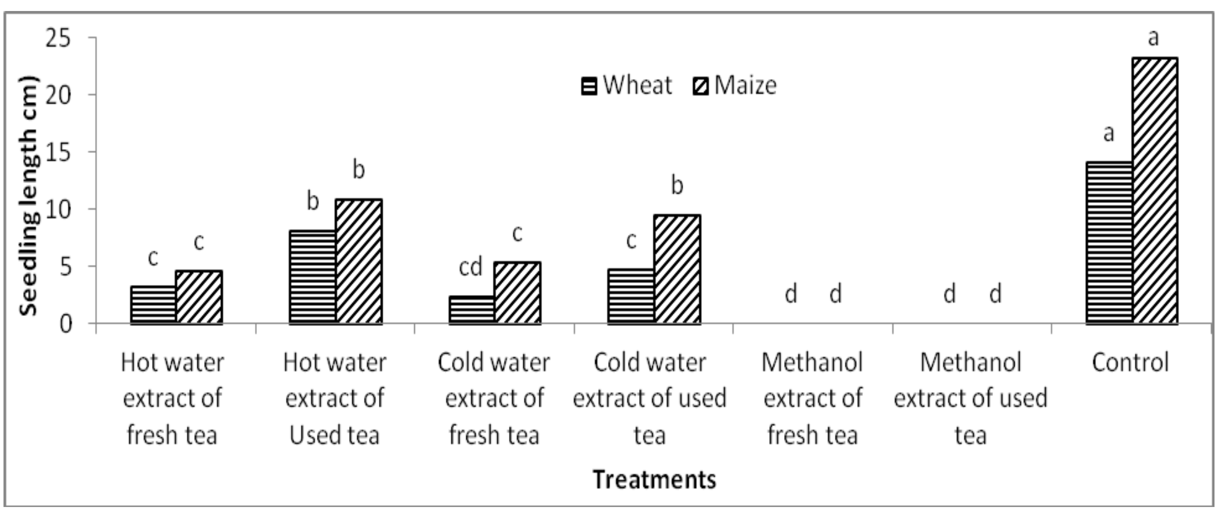

Fig. 6. Effect of tea extract on the seedling length of wheat and maize.

Fresh Biomass (mg) plant^{-1}

Data revealed significant (P≤ 0.01) differences among plant fresh weights as affected by different tea extracts. The mean values for wheat regarding plant fresh weight (Fig.7) illustrated that maximum fresh biomass plant^{-1} was recorded in hot water extract of used tea (185.3 mg) closely followed by the plants grown in control (89.0 mg) treatment and those grown in hot water extract of fresh tea (60.0 mg). Plants grown in cold water extracts of fresh and used tea behaved alike producing minimum biomasses of 37.0 mg34.3 mg, respectively. The mean values for maize (Fig.7) regarding fresh biomass followed almost a similar pattern as those of wheat. Maximum fresh biomass was recorded in hot water extract of used tea (422.0 mg) closely followed (at par) by fresh biomass acquired by the plants in control (355.3 mg) treatment. The rest of the three treatments, i.e. cold water extract of used tea, cold water extract of fresh tea and hot water extract of fresh tea were at par with each other producing lesser biomasses of 239.3 mg, 183.3 mg and 170.0 mg. In a similar study, Abdulghani (2012) treated the soil with black tea wastes with application of 0%, 2%, 4%, 6%, respectively and planted barley seeds in which 4% treatment showed high dry biomass of seedling. It interesting that the tea extracts, though inhibited germination, had a positive effect on the plant fresh biomass. Some treatments (hot water extract of used tea) not only produced fresh biomasses at par with control but were even slightly higher. These were observed in both wheat and maize. This depicts that tea extracts could be equally efficient for other crop or plant species as well. It is obvious from the results that tea extracts inhibited seed germination but at the same time increased the vegetative growth (in the form of fresh biomass in this case). It is worth further investigation to understand this mechanism of inhibition and enhancement of germination and growth.

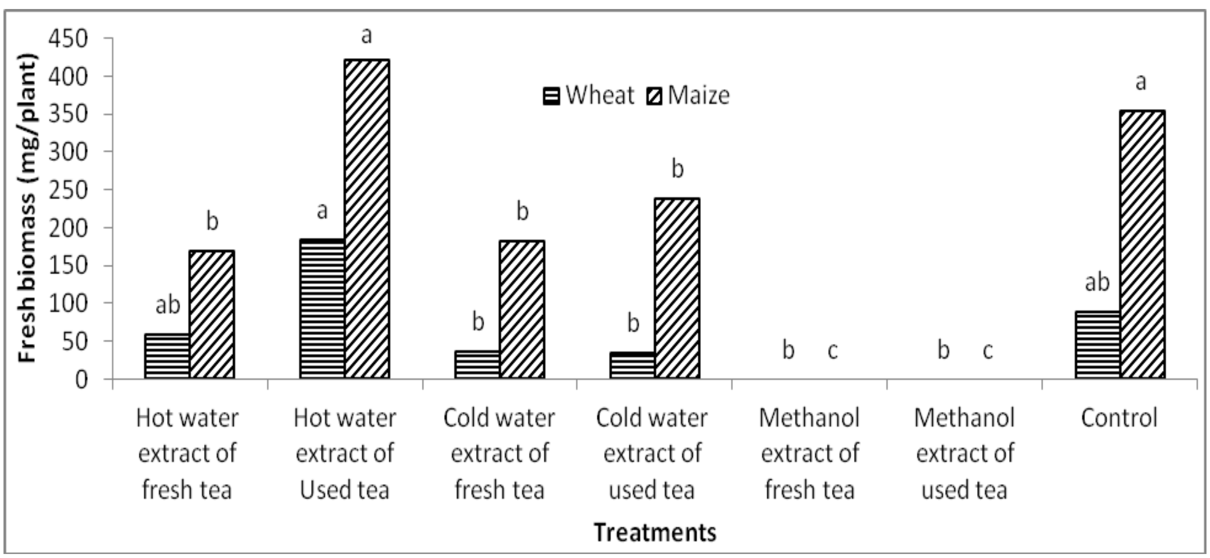

Fig. 7. Effect of tea residue extracts on the fresh biomass of wheat & maize seedlings.

Conclusion

Tea residue extracts might have strong biological activity in the field of agriculture. Aqueous extract of tea residues significantly affected germination of wheat and maize. Toxic and inhibitory effects were shown by all treatments with profound effects of methanol extracts. On the other hand, some positive effects of vegetative growth enhancement were also observed, More studies of pot and field experiments are suggested for meaningful conclusion. Residues of tea may contain some growth promoting and bio-active substances. It would be a luminous direction to proceed in order to improve agricultural sustainability, environmental safety, food security, resource conservation and economic stability. More studies are needed to fully explore the use of tea waste in agriculture and organic food production.

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