THE INFLUENCE OF SOME MEDICINAL PLANTS ON SEED EMERGENCE OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

The impact of medicinal plant extracts on the seed emergence of chickpeas was examined under *in vitro* conditions through a randomized design method with three replicates (0.5g, 1g, and 1.5g) with six Petri plates each. For this purpose, the aqueous flower extract of *Achillea santolina* (Boh-e-madran), the leaf extract of *Melissa officinalis* (Balm), *Melia azedarach* (Wild neem), and flower and leaf extracts of *Mentha piperita* (Peppermint), were applied on chickpea (*Cicer arietinum* L.) seeds. The results were obtained after three different time durations, 24 hrs, 48 hrs, and 72 hrs, respectively. The study had shown that these medicinal plants had inhibitory effects on seed emergence (Plumule and radicle), which were compared with the control that was treated with distilled water for the positive correlation. The maximum inhibitory effect was recorded in the leaf and flower extracts of *Mentha piperita* and the minimum was in *Melissa officinalis*. To carry out the previous medicinal indications, the phytochemicals of two medicinal plants, *Mentha piperita (leaf)* and *Achillea santolina* (flower) were also examined by following the soxhlet apparatus technique. The results had shown the presence of saponins, alkaloids, flavonoids, tannins, and fixed oil in both plants, phenol in *Mentha piperita* only while the reducing sugar was present in the flower extract of *Achillea santolina* and absent in the leaf of *Mentha piperita*. The study summarized the presence of medicinal properties in plant extracts that worked as an inhibitor for chickpea seeds. Two of these medicinal plants illustrated some allopathic properties which were confirmed through the soxhlet method. This research contributed to the importance of local medicinal plants that could be used for various reasons as natural stimulators and inhibitors.

Keywords: allelopathic, chickpea, soxhlet, reducing sugar.

INTRODUCTION

Pakistan has various climatic zones, is rich with unique biodiversity of 6000 higher plant species, and lies between 60°55' to 75°30' E longitude and 23°45' to 36°50' N latitude. Many research works have been conducted with medicinal plants having ethnobotanical importance. The local and indigenous communities across the country have their traditional knowledge regarding the use of plants for different purposes, However, this information has been transferred from one generation to another generation that these plants are used for the treatment of all kinds of diseases from skin related disease to kidney problems. There are hundreds of identified medicinal plants which have been used in modern medicines for the treatment of different diseases, e.g., cough,

asthma, fever, malaria, chest diseases, flu, colds, headache, skin issues, nausea, tumour, ulcer, heart attack, inflammation, swelling, etc. Most of these species are collected from wild sources, which increased the demand for medicinal plants based on drugs and by pharmaceutical industries across the world (Ishaque & Shahni, 1998). The allelochemical properties are present in different plant parts like shoots, leaves, flowers, fruits, roots, and seeds. They are extracted from plant residues through different methods like exudation, volatilization, and decomposition. However, the major chemicals are phenolic compounds that have an impact on plant growth and seed germination, Low concentrations of phenols in plants exhibit stimulatory effects while high concentration of phenols reduces germination (Hegab *et al*., 2008; Ghareib *et al*., 2010; Molisch, 1937). These compounds are natural herbicides and pesticides which have been used for high-yield production.

Allelopathy is observed as a direct or indirect interaction when one plant can release certain metabolites and chemicals which influence the physiological processes of adjacent plants, and these effects may be beneficial or harmful to adjacent plants (Ravindra *et al*., 1977; Rice, 1984; Williamson, 1990). Proper use of medicinal plants will increase the efficacy of the medicine, which is the main focus of medicinal plant practitioners, who believe that there is a relation between plants with medicinal qualities and humans (Juden, 2003)

There is much research, mainly emphasizing the allelopathic properties of medicinal plants on other plants' growth and germination, such as *Cannabis sativa*, which is an annual crop and well recognized because of its property in controlling pests, was used against *Lactuca sativa* L, where the allelopathic activities of this plant were examined (Ranalli, 1999).

In this study, the seeds of Cicer known as chickpea (*Cicer arientinum*) were selected (Rehm, 1994). Chickpea also called chana or Kabuli chana, is one of the earliest cultivated legumes from the family Fabaceae, and its seeds are rich in protein (Bell, 2014). To evaluate the allelopathic influence of medicinal plants on chickpeas, four plants from Balochistan were opted for their medicinal values by local medicinal plant practitioners across Quetta, Balochistan. Hence, the peppermint (*Mentha piperita*) from the family Lamiaceae, and *Melia azedarach* from the family Meliaceae, were selected (Floyd, 1989). The leaves of this tree serve as a natural insecticide and a diluted concentration of leaves of *Melia azedarach* was used in the past to activate uterus relaxation (Russell *et al*., 1997). The third plant was *Melissa officinalis*, commonly known as balm from the family Lamiaceae, while the fourth one was *Achillea santolina*, from the family Asteraceae, which is a perennial herb with erect stems, and its aerial parts such as flowers and leaves contain essential oil used by indigenous people to cure intestinal disorders and diarrhoea (Shazyl *et al*., 2004). Moreover, these plants are the most recognized plants among locals because of their medicinal properties, hence the main objective of this present study is to evaluate the allelopathic activities of these medicinal plants, *Achillea santolina* (Boh-e-madran), *Melissa officinalis* (Balm), *Melia azedarach* (Wild neem) and *Mentha piperita* (Peppermint), on chickpea (*Cicer arietinum* L.)

MATERIAL AND METHODS

1. Plants collection: The two plants parts of *Mentha piperita* and *Melia azedarach* were collected from the Sardar Bahadur Khan Women's University, Quetta Campus. The raw samples contained the leaves and flowers of these two plants. The other two plants were brought from

different places: *Melissa officinalis* was brought from Bela (District Lasbella), while *Achillea santolina,* and Chickpea seeds were brought from the local medicinal shop in Quetta.

2. Extract Preparation

Digital balance was used to weigh the dry material of these medicinal plants in three different values, 0.5g, 1g, and 1.5g. Dried materials were crushed with the help of mortar and pestle and mixed well in 50 ml distilled water separately. These extracts were capped for 24 hrs, 48 hrs, and 72 hrs at room temperature, and then the filtrates were collected, following Mishra (2014)

3. Seeds soaking

In three replicates, six petri plates were arranged with five seeds of chickpea in each, covered with a fine layer of cotton and were kept at an equal distance and soaked in 5 ml of filtrate. The seed emergence was recorded after 24 hrs, 48 hrs, and 72 hrs separately.

Phytochemical analysis of *Mentha piperita* and *Achillea santolina*

A. Plant material:The fresh leaf material of *M. piperita* and the flower of *A. santolina* were collected, washed, and air-dried at room temperature and then ground with an electrical blender to reduce it to powder form by following the method of Yadav & Agarwala (2011).
B. Extract preparation in Soxhlet apparatus: In 500ml of ethanol (C_2H_6O), 20 g of powder plant material was mixed and prepared by using the soxhlet apparatus for 2 hours. After that, residues were collected and used for further phytochemical analysis, following Yadav &Agarwala (2011).
C. Qualitative analysis.
   Test for saponins: 0.05g of powder plant material was dissolved in 20 ml distilled water by continuous shaking for 15 minutes for the formation of the layer, after that the results were obtained by following the method of Mustafa (2015).

1. Test for alkaloids: for the determination of alkaloids, 5ml of the prepared extract was added to 1-2 ml of Hager's solution, and the result was calculated, following Mustafa (2015).
2. Test for flavonoids: 5 ml of test solution was added into 5 ml of NH3, and to that 4 drops of H_2SO_4 were added. After that, the result was obtained by following the procedure of Mustafa (2015).
3. Test for phenol: The dilution of 0.05g of plant sample was made in distilled water at 100 °C, after cooling down the solution, 3 ml of 10% Pb $(CH_3COO)2$ was added to the diluted solution. Then the results were calculated by following the method of Mustafa (2015).
4. Test for tannins: 0.05 g of dried plant material was boiled at 100 °C in 20 ml of distilled water. After settling the boiled solution, 1-3 drops of 0.1% FeCl3 was added, then the result was obtained immediately by following the method of Mustafa (2015)
5. Test for fixed oil: 3 ml extract passed between two filter papers to observe the oily appearance on the filter paper. The test was performed by following the method of Mustafa (2015).
6. Test for protein: In 5 ml of aqueous filtrate, 3 drops of ninhydrin solution were added for the determination of protein in the test samples. Then the

result was noted by following Mustafa (2015).

7. Test for steroids: 0.05 g of plant material was mixed into 20 ml of ethanol, then 2 ml of $C_2H_4O_2$ and 2 ml of H_2SO_4 were added, and then the results were calculated following Mustafa (2015).
8. Test for carbonyl group: in 4 ml of plant extract, 2 drops of aldehyde was added, and then the solution was boiled in the water bath at 100° C. The confirmation of the compound was observed by following Mustafa (2015)
9. Test for reducing sugar: In 2ml of plant extract, 3 to 4 drops of Benedict's solution were added, and then the solution was placed to boil at 100° C. The results were observed following Mustafa (2015)

RESULT

Based on the research study, the data on the influence of medicinal plant extract on seed emergence and germination of chickpea (*Cicer arietinum* L) were examined. The extract was prepared from the leaf and flower parts of *A. santolina* (Boh-e-madran), *M. piperita* (Peppermint), leaf extract of *M. officinalis* (Balm), and *M. azedarach* (Wild neem). The results of three different replicates (0.5, 1, and 1.5 g) and treatment which showed the significant effect on seed emergence and germination rate of chickpea seeds The calculated values of chickpea seed emergence were treated with the leaf and flower extract of these medicinal plants, which confirmed the inhibitory effect on seeds because of their allelopathic properties. The obtained results had shown, the reduction in seed emergence at their initial stage to limit the plumule and radical growth. After three different time frame observations, this study confirmed the inhibition in seed emergence in comparison to the control, which was treated with tap water.

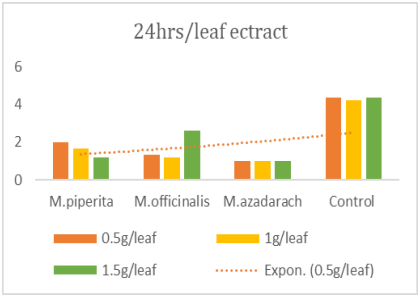

Figure 01: Influence of leaf extracts of *M .piperita, M .officinalis,* and *M. azedarach*on seed emergence of chickpea after 24 hrs.

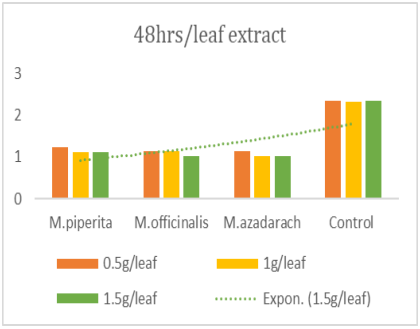

Figure 02: Influence of leaf extracts of *M . piperita, M . officinalis,* and *M. azedarach* on seed emergence of chickpea after 48 hrs.

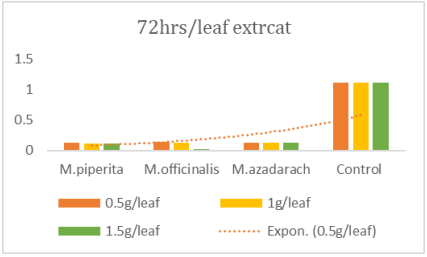

Figure 03: Influence of leaf extracts of *M. piperita*, *M. officinalis*, and *M. azedarach* on seed emergence of chickpea after 72 hrs.

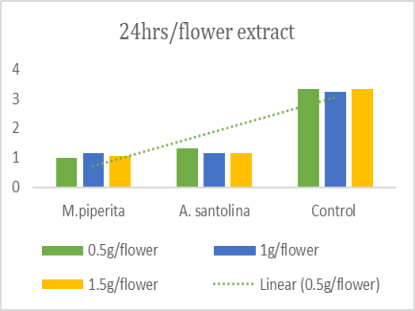

Figure 04: Influence of flower extracts of *M. piperita* and *A. santolina on* seed emergence of chickpea after 24 hrs.

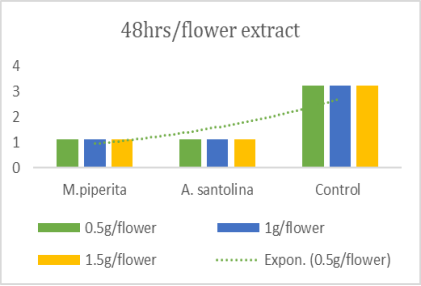

Figure 05: Influence of flower extracts of *M. piperita* and *A. santolina* on seed emergence of chickpea after 48 hrs.

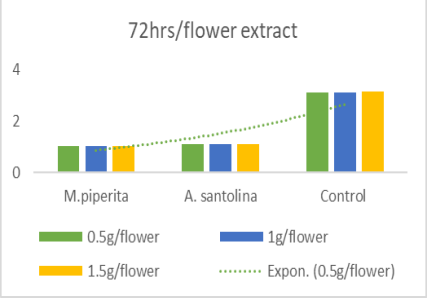

Figure 06: Influence of flower extracts of *M. piperita* and *A. santolina on* seed emergence of chickpea after 72 hrs.

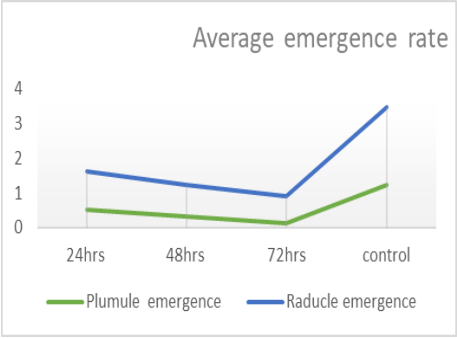

Figure 07: Average seed emergence rate (plumule & radicle) of chickpea (*Cicer arietinum*) influenced by leaf and flower extracts of *M. piperita*, *M. officinalis*, *M. azedarach* and *A. santolina*.

DISCUSSION

In this study, the seed germination of chickpea (Cicer arietinum) was examined by different aqueous leaf and flower extracts of medicinal plants such as M. piperita (leaf/flower), M. officinalis (leaves), M. azedarach (leaves), and A. santolina (flower). For this purpose, the results were obtained from three replicates result after three different time periods observation. The emergence rate was observed on the basis of plumule and radicle disclosure after 24 hrs, 48 hrs, and 72 hrs along with control, respectively. Hence, this study confirmed the inhibitory effect of these medicinal plant extracts on chickpea seeds, which had shown the presence of certain phytochemicals in these selected plant parts. However, phenols are the most important allelochemicals which were documented and considered to be the major compound to limit the ability of seed growth and germination (Chon et al., 2002) Therefore, these medicinal plant extracts exhibited the

growth reduction by limiting seed emergence, which is accepted through different research literature that the excessive concentration of phenolic compounds are responsible for the sharp growth reduction during germination, while low concentration has a stimulatory impact on plant growth and seed germination. In Cannabis sativa, some well-known compounds were identified such as terpenoids (Hegab et al., 2008); Ameh et al., 2010): Ghareib et al., 2010), which deteriorated the physiological functioning of seeds by disorientating the membrane to limit the energy supply for the growth. Hence, this could be a reason that inhibited more seed emergence of chickpea after 48 and 72 hours, in comparison to 24 hours. The plants release more allelochemicals with the passage of time denatured the important cells and their functioning restricted the transfer of ATP. In this study, all plants reduced the seed germination by limiting the plumule and radicle outgrowth in comparison to 24 hrs. However, M. piperita also exhibited a strong smell compared to the other three plant extracts which had shown the presence of certain secondary metabolites.

Seeds Emergence

The chickpea seeds were imbibed with leaf and flower extract of the tested medicinal plants such as M. piperita, A. santolina, M. azedarach, and M. officinalis which were responsible for the procrastination and inhibited the seed growth in comparison to control (0 treatment). The results concluded the inhibitory effect on seed emergence in all three replicates treated with the leaf and flower extracts. However, the leaf extract of these plants possessed more inhibitory effects than a flower; that might be due to the interference of these phytochemicals in enzyme activity and irregularity in metabolism (Mahmoodzadeh, 2015; Oyun, 2006). Moreover, these phytochemicals contained, phytotoxins which changed the structure of the plasma membrane by denaturing it when the seed was soaked in the extract. A correlation was observed between the high concentration of extract and the inhibition of seed germination rate. Therefore, in this study the allelochemicals were identified as responsible to inhibit the metabolic processes by limiting the plant's potential to target the growth mainly for the ATP synthesis, this was required for the seed to grow (Mahmoodzadeh, 2015; Demos et al., 1974).

Phytochemical analysis

These local medicinal plants are having utilization for different treatments, particularly for cough, flu, skin disease, etc by local communities. These plants contain certain phytochemicals which may influence seed growth and emergence by stimulating or inhibiting at their initial stages. Hence, to study the phytochemical properties of the leaves of Mentha piperita and the flower Achillea santolina, the soxhlet apparatus was used. This study detected various phytochemicals in the leaves and flower parts of Mentha piperita and Achillea santolina. This had shown the high rate of steroids and phenols present in the leaves of Mentha piperita while the same absent in the flowers of Achillea santolina. However, saponins, alkaloids, flavonoids, and tannins were present in both plant parts, while protein and carbonyl groups were absent in both. Reducing sugar was present in the flower of Achillea santolina while absent in the leaves of Mentha piperita, respectively (Mustafa, 2015).

Phytochemicals Analysis

Phytochemicals	Saponins	Alkaloids	Flavonoids	Phenol	Tannins	Fixed oil	Proteins	Steroids	Carbonyl group	Reducing sugar
M. piperita	+	+	+	+	+	+	-	+	-	-
A. santolina	+	+	+	-	+	+	-	-	-	+

Present = (+), absent = (-)

Table 01: the presence of certain phytochemicals in the flower extracts of *M. piperita* and *A. santolina* which were performed by soxhlet apparatus.

CONCLUSION

There are certain contents of phytochemicals present in the flower and leaf extracts of the medicinal plants Achillea santolina (Boh-e-madran), Melissa officinalis (Balm), Melia azedarach and Mentha piperita, that may be causing inhibition of the metabolic activities of chickpea seeds in vitro Bio-assay test. The study disclosed the emergence potential of chickpea seeds, which was noticed between non-treated and treated seeds with flower and leaf extracts. However, the calculated data revealed the statistical significance that displayed the presence of allelopathic effect on seed emergence.

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