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Allelopathic effects of *Artemisia fragrans* Willd., *Tanacetum chiliophyllum* Sch.Bip. and *Teucrium polium* L. on establishment of *Medicago scutellata* (L.) Mill in degraded shrublands

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ABSTRACT

In pot culture, we investigated the allelopathic effects of 3-donor plants [*Artemisia fragrans* Willd., *Tanacetum chiliophyllum* (Fisch. & E.Mey. ex DC.) Sch.Bip. and *Teucrium polium* L.] on establishment of sown *Medicago scutellata* (L.) Mill. in shrubland invaded by grasses. We used 4-concentrations (0, 5, 10 and 20 %) of aqueous extracts of 3 -donor plant spp. (*A. fragran*, *T. chiliophyllum* and *T. polium*) on germination and seedlings growth of recipient *M. scutellata*. The seeds germination of *M. scutellata* decreased with increasing concentrations of aqueous extract. The *T. chiliophyllum* 5 % aqueous extract was least inhibitory to seeds germination of *M. scutellata*. While the *A. fragrans* 20 % aqueous extract was most inhibitory and significantly delayed the seeds germination than control. Seed vigour index in *T. chiliophyllum* aqueous extracts was significantly higher than other treatments and control. *T. chiliophyllum* 5 % aqueous extract stimulated the root length. The *A. fragrans* aqueous extracts were most inhibitory to plant biomass, leaf number and leaf area. The inhibitory effects of aqueous extracts of all donor plants on *M. scutellata* seeds germination and seedlings growth increased with increasing aqueous extracts concentrations i.e. concentration dependent. The 20 % aqueous extract of *A. fragrans* was most inhibitory, while, 5 % aqueous extract of *T. chiliophyllum* were least inhibitory. The inhibitory alleloopathic effects of donor spp ranked as *A. fragrans*, *T. polium* and *T. chiliophyllum.* Therefore, it is necessary to consider the allelopathic effects of introduced species on rangeland plant spp. Therefore, for sowing of *M. scutellata*, the under story canopy of *T. chiliophyllum* is best. Moreover, the sites with low or moderate density of *T. chiliophyllum* and *T. polium* have the highest chances of success to reclaim shrubland. Whereas in areas with abundant *A. fragrans*, the sowing should be done cautiously due to its very drastic inhibitory effects. Additionally, the research on success of other forage species to restore the *A. fragrans* dominated rangelands is suggested.

Key words: Allelopathy, aquatic extract, *Artemisia fragrans*, growth, *Medicago scutellata*, rangeland species, seed germination, *Tanacetum chiliophyllum*, *Teucrium polium.*

INTRODUCTION

The allelopathic interactions controls the species distribution and abundance within plant communities, hence, these are important for the success of newly introduced plants in rangelands (14,26). Allelopathy plays a significant role in plant dominance, establishment of new-introduced species, succession and the formation of plant communities (10,52). In

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the semi-arid regions, low vigour of germinated seeds of new spp. and their growth may be related to allelopathic effects (5).

Thus, success of seedling establishment depends on seeds germination power and seedlings growth under environmental stresses, where allelopathy plays greater role in success or failure of rangeland rehabilitation (18).

Allelopathic potential of different plants depends on their phytochemicals contents (34,50). Wang *et al.*, (44) reported that in vegetation succession studies in typical grasslands, the allelopathy of *A. sacrorum* shapes the botanical composition of grasslands on Yunwu Mountain in 4-dominant grassland species. The *Thymus kotschyanus* allelopathically inhibited the germination of sown *Agropyron elongatum* and *Bromus inermis* in rangeland restoration (33). Cipollini and Bohrer (7) studied the comparative direct and indirect allelopathic effects of leaf aqueous extracts of 5-species in laboratory and found that the effects of each donor specie varied with target species and life stage. Hou *et al*., (17) reported that the allelopathic potential of invasive weed was stronger in the soil from the later successional stage than from the earlier successional stage in forest community in Southern China. Xu *et al.*, (46) determined the allelopathic effects of fescue (*Festuca arundinacea*) variety 'Millennium2' leaf aqueous extracts on 10 fescue varieties, they reported that physiological indices of fescue varieties had variable responses to the leaf aqueous extracts.

In the most rangelands of Iran, the valuable palatable species are being replaced by unpalatable grass species due to high grazing pressure. The invasion of palatable plants by unpalatable annual grasses is main problem leading to the degradation of the semi-arid shrublands of Northwest Iran. The invasive species have superior competitive ability than native species. Some annual grasses (*Bromus tectroum*, *B. inermis*, *Poa bulbosa*) have hairy surface of roots and have high potential for reproduction, thereby overcome the native species (15). Due to the predominance of annual grasses of short life and flammability in rangelands, the risk of fire increases. Additionally, invasive plants significantly reduces the stabilities of microbial communities and food webs and cause severe ecological problems (42). The rangelands invaded by invasive grass species need to be restored by sowing new useful appropriate legume species to change the composition of plant community. In such areas invaded by invasive grasses, there is an urgent need to increase the legume species in combating the grass invasion. Introduction of legumes (*e.g. M. scutellata*) as potential resistance specie, it provides palatable fodder and fixes atmospheric nitrogen in soil can be appropriate specie for this area (25).

(i). *Teucrium polium* is a perennial sub-shrub native to the semi-arid regions. Its flowers are small pink to white. Its leaves have significant ingredients used in many traditional medicine prescriptions and cooking. It is used in different pathological conditions, including inflammations, gastrointestinal disorders, diabetes, rheumatologic diseases and diabetes in Iranian folk medicine (Figure 1).

(ii). *Tanacetum chiliophyllum* is a perennial subshrubs, has more branching stems from rhizomes. It is aromatic and has high medicinal value. Its essential oil is rich in 1,8-cineole, camphor and linalool, hence, used for the treatment of several diseases in traditional medicine system. Its raw material has great scope in nutraceutical, cosmeceutical, and pharmaceutical areas (Figure 1).

(iii). *Artemisia fragrans* is a perennial shrub and contains essential oils. It has strong aromas and bitter tastes due to terpenoids and sesquiterpene lactones, which discourage herbivory. It has green silver leaves and yellow flowers, widely distributed in Iran, Azerbaijan, Russia and Turkey. The plant and its essential oil (EO) has antibacterial and antioxidant activities, hence, used in traditional medicine and in pharmacological and cosmetic industries (Figure 1).

(iv). ***Medicago scutellata*** is a legume plant used for rangeland restoration. It forms a symbiotic relationship with bacterium *Sinorhizobium meliloti* and fixes atmospheric nitrogen in soil. It is also stress tolerant and excellent forage crop for livestock. Its seeds are rich in trypsin inhibitor, hence, is potentiator of cisplatin-mediated trypsin inhibitor, as a plant-derived anticancer agent for human breast and cervical cancers (Figure 1).

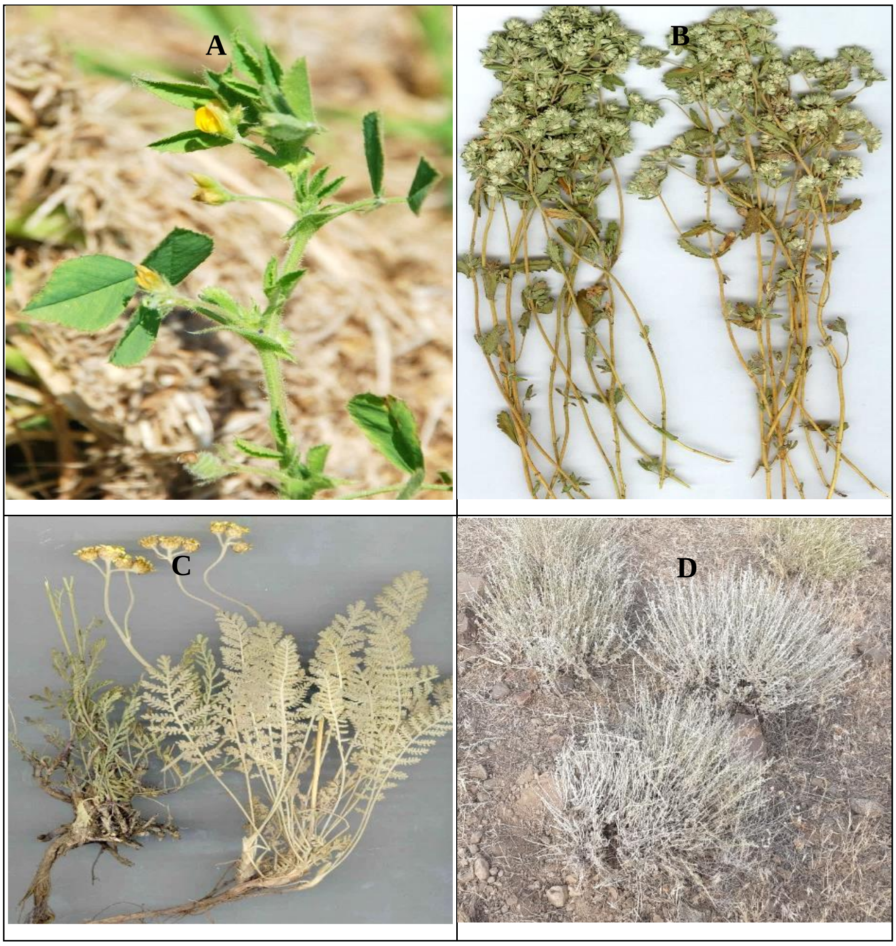

Figure 1. Medicinal plants of *M. scutellata* (A) *T. polium* (B), *T. chiliophyllum* (C) and *A. fragrans* (D)

The allelopathic interactions of *M. scutellata* and the medicinal shrubs (*A. fragrans*, *T. polium* and *T. chiliophyllum*) abundant in semi-arid rangelands of northeast Iran have been least studied (51). There is need to ensure that the *M. scutellata* seeds (i) germinate normally, (ii) grow well in the degraded rangelands under the allelopathic stress and

(iii) survive and naturally reproduce to restore the diversity and stability of community. Therefore, to minimise the allelopathic risk and success in seed sowing, it is necessary to select the suitable plant's understory and its appropriate plant density. The dry plants of these spp (*A. fragrans*, *T. polium* and *T. chiliophyllum*) are sold at 1.5-2 US $/kg as herbal drugs. This study aimed to investigate (i) the effects of aqueous extracts of *A. fragrans*, *T. chiliophyllum* and *T. polium* on seed germination and plant growth of annual *M. scutellata* and (ii) finding the ideal understorey canopy of shrubs and their density for sowing of *M. scutellata* legume plants.

MATERIALS AND METHODS

I. Study Site

The studies and sampling were done in Eslami Island (37°43′14.13″ to 37°56′09.55″ N, 45°24′45.90″ to 45°35′13.46″ E) in northwest (East Azerbaijan province) Iran (Figure 2), 80 Km from Tabriz. The topography is mountainous, elevation 1300-2000 asl. It is semi-arid area, mean annual precipitation of 331 mm and temperature 11.3 ºC. Natural vegetation is representative of semi-steppe bioclimatic region mixed brush shrubland and forbs with occasional perennial grasses. The soil is loam. This study was done from June to August 2019.

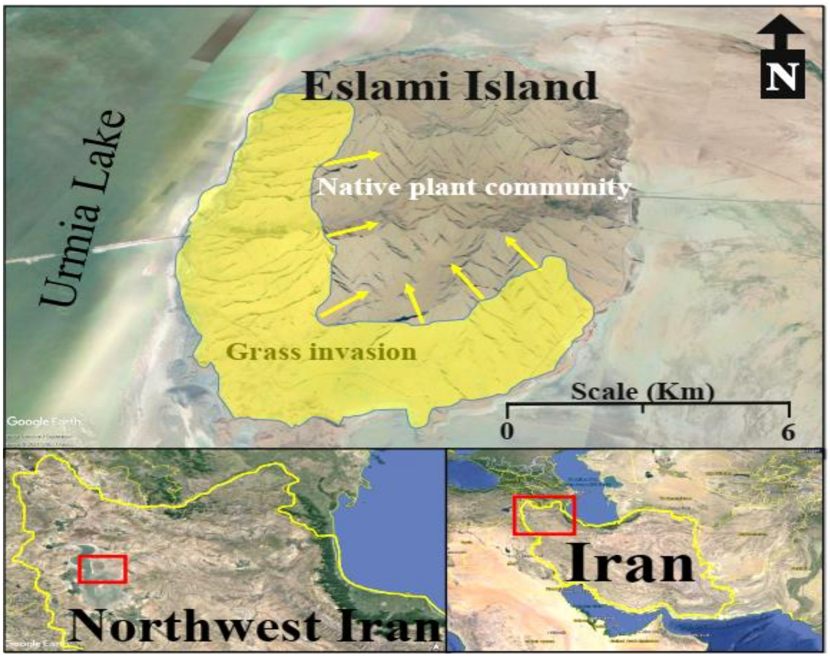

Figure 2. Study Site: Eslami Island of Urmia Lake, northwest Iran (37°43′14.13″ to 37°56′09.55″ N, 45°24′45.90″ to 45°35′13.46″ E)

In this area, the annual grass species have dynamic invasion status into the native shrub species. The plant species includes dominant shrubs (*A. fragrans*, *T. polium* and *T. chiliophyllum*) and some forbs, these are invaded by grass species (Figure 3).

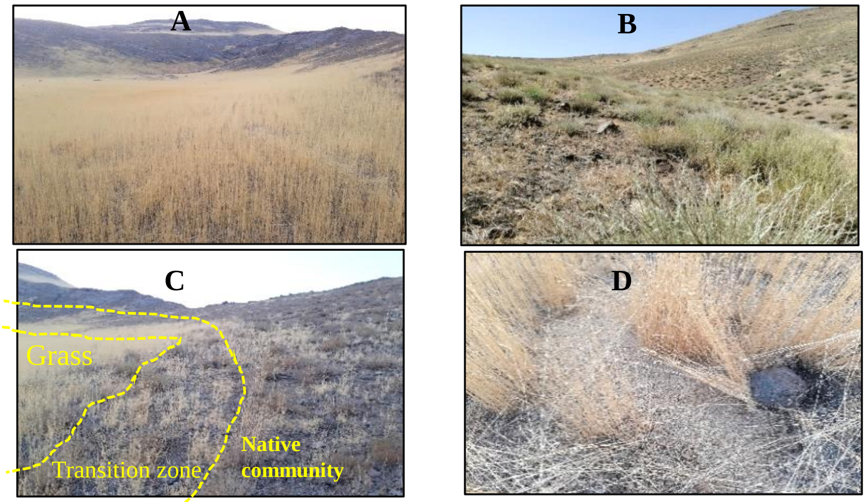

Figure 3. Study sites. **A:** Native plant community; **B:** Site invaded by grass; **C**: Close view of native community, showing barrier between two communities, **D:** High seed yield of grasses.

II. Experimental

Experiments were done in environment controlled greenhouse, because of allelopathy influences and interspecific competition in the biological system (8).

In late May 2019, the shoots of donor plants (*A. fragrans*, *T. chiliophyllum*, and *T. polium*) were harvested from the rangeland. These were dried at room temperature and ground to fine powder. Two hundred g powder of each plant specie was mixed with 1000 mL distilled water (1: 5 ratio) and kept overnight. It was stirred for 1.0 h on shaker (160 rpm) and filtered through filter paper (Whatman No. 1) to obtain the crude aqueous extract. To simulate different shrubs densities at the sites, 4 crude aqueous extract concentrations, i.e. (0, 5, 10 and 20 %) were prepared with distilled water, respectively, representing the low, moderate and high density of shrubs. These aqueous extracts were then stored in refrigerator at 4 °C until use. These aqueous extracts were used to irrigate the *M. scutellata* sown pots @ 300 mL per pot twice a week. Before sowing, seeds of *M. scutellata* were sterilized using sodium hypochlorite (5 %) solution for 15 min and then washed several times with distilled water and dried (14). On 1 June 2019, 20-seeds of *M. scutellata* as recipient plant were sown per pot (15 cm dia and 20 cm depth, filled with 3 kg sandy loam soil). The pots were kept in greenhouse at 25 °C for germination and growth.
Experimental Treatments: The experimental treatments consisted of two factors: (i). Donor spp 3 (*A. fragrans*, *T. chiliophyllum* and *T. polium*) and (ii). aqueous extract concentrations 4 (0, 5, 10, 20 %). The treatments were replicated 5-times in Complete Randomised Design.

III. Germination indices: Two weeks after sowing (14 June), the seeds were considered germinated when > 2 mm long radicle emerged from the geminating seeds (9). The Germination (%), Germination Rate, Mean Germination Time and Seed vigour Index (9) were calculated as under:

(i). Germination (%): It was calculated using the formula: $GP = \frac{n}{N} \times 100$
Where, GP: Germination (%), n: Number of germinated seeds, N: Total number of seeds.
(ii). Germination Rate: It was calculated using the formula: $GR = \sum_{i=0}^{d} \frac{ni}{di}$
Where, GR: Germination Rate, ni: Number of seeds germinated on day I, di: number of days since the start of the experiment.
(iii). Mean Germination Time: It was calculated using the formula: $MGT = \frac{\sum ni \times di}{n}$
Where, MGT: Mean germination time, n: the total number of seeds germinated on the last day of counting, ni: number of seeds germinated on day I, di: number of days since the start of the experiment.
(iv). Seeds vigour Index: It was calculated using the formula: $SVI = \frac{Gp \times MSH}{100}$
Where, SVI: Seeds vigour Index, MSH: Mean length of seedling (root + stem) in mm, Gp: Germination (%).

IV. Growth

On 15 June, the seedlings were thinned to one seedling per pot to grow till two months i.e. harvest. On 15 August, the leaf number were counted in each plant and leaf area of 5-leaves was measured with Leaf Area Meter Model 1.3. The plants were partitioned in root and shoot, their fresh weight was recorded and length was measured by ruler.

V. Isolation of essential oils and GCMS analysis

The essential oils were extracted for 3 h, from 100 g dried shoots of *A. fragrans*, *T. chiliophyllum* and *T. polium* plants by hydro-distillation using a clevenger type apparatus. The collected oil was dried over anhydrous sodium sulfate and stored in refrigerator at 4 °C until analysis. Chemical compositions of essential oils was analyzed by an Agilent 7890A Network GCMS system pooled with Agilent 5975C Network with Triple-Axis mass detector. The GCMS analysis was carried out on the Agilent 7890A Network GCMS system equipped with a split less model injector (with 1.0 µm volume and 250 °C temperature). Carrier gas was helium with a flow rate of 1.1 mL/min and the capillary column used was HP 5-MS (30 m×0.25 mm, film thickness 0.25 µm). The column pressure was fixed to 56054.38 Pa. The oven temperature initially was kept at 50 °C for 2 min after injection and then increased to 250 °C with a rate of 6 °C/min heating ramp and kept constant at 250 °C for 4 min. The ionization voltage and mass range were 70 eV and 34-500 m/z respectively. The 280 °C and 250 °C were used as anion source and interface temperatures, respectively. Constituents of the essential oils were recognized based on their retention time and mass spectra pattern with related available data or with Wiley library and literature. Each compound (%) were calculated from the given GCMS peak area and these data were used for quantification purposes.

VI. Statistical analyses

The Inhibition/Stimulation effects (%) of aqueous extract of 3- donor species and 3 doses were calculated as under:

$$\text{Inhibition/Stimulation (\%)} = \left(\frac{pvt - pvc}{pvc}\right) * 100$$

Where, pvt; Parameter value in treatment, pvc: Parameter value in control.

RESULTS AND DISCUSSION

I. Allelochemicals in donor species

In total, 36 different compounds were identified in essential oils of 3-test shrubs (*A. fragrans, T. chiliophyllum* and *T. polium*) using GC-MS analysis (Table 1). The 1,8-cineole (25 %), camphor (15 %), α-thujone (9.5 %), and 4-terpineol (9.5 %) were the main constituents in EO of *A. fragrans*. In EO of *T. chiliophyllum* camphor (19 %), borneol (18.5 %) and 1,8-cineol were the main compounds. The essential oil of *T. polium* contained limonene (17 %), muurolol (13 %), α-pinene (10.75) and β-pinene (8 %). The majority of detected compounds were monoterpenes and sesquiterpenes (simple and oxygenated derivatives), i.e. these are large portion of essential oils.

Table 1. Compositions (%) of essential oils of *A. fragrans*, *T. chiliophyllum* and *T. polium* plants

No.	Compounds	RT	RI[a]	RI[b]	*A. fragrans*	*T. chiliophyllum*	*T. polium*
1	Santene	5.7	880	879	1.6	-	-
2	Santolina triene	6.0	894	898	1.7	-	-
3	α -Pinene	6.4	917	919	-	1.2	10.75
4	Artemisia triene	6.5	920	924	3.0	-	-
5	**Camphene**	**6.7**	**933**	**934**	**3.5**	**2.5**	**-**
6	Sabinene	7.4	971	961	0.4	1	3.2
7	β –Pinene	7.6	978	976	-	0.70	8.0
8	β -Myrcene	7.7	981	977	-	-	3.5
9	Yomogi alcohol	8.0	999	995	8.6	-	-
10	**α -Terpinene**	**8.3**	**1017**	**1015**	**-**	**0.75**	**-**
11	p-Cymene	8.4	1021	1018	-	4.5	-
12	**1,8-Cineole**	**8.5**	**1028**	**1028**	**25**	**17.5**	**-**
13	Limonene	8.5	1030	1029	-	-	17
14	**γ –Terpinene**	**9.1**	**1060**	**1056**	**0.5**	**2.7**	**1.5**
15	Artemisia alcohol	9.5	1082	1090	4.5	-	-
16	Linalool	9.8	1104	1103	-	1.5	-
17	β –Thujone	10.0	1112	1111	7.5	2.5	-
18	α –Thujone	10.0	1114	1115	9.5	-	1.25
19	Trans-p-Menth-2-en-1-ol	10.8	1155	1160	-	2.25	-
20	Camphor	10.9	1160	1157	12	19	12
21	Borneol	10.9	1163	1170	-	18.5	-
22	Pinocarvone	11.0	1168	1171	-	4.5	6
23	4-Terpineol	11.2	1178	1182	9.5	1.5	1.2
24	Myrtenol	12.4	1234	1239	1.4	-	-
25	Piperitone	12.8	1253	1253	-	2.5	-
26	Bornyl acetate	13.1	1261	1264	5.5	3.2	-
27	Isopiperitenon	13.3	1272	1265	0.2	-	-
28	Trans-Verbenyl acetate	13.7	1292	1292	-	-	0.5
29	Carvacrol	14.2	1307	1308	-	0.75	-
30	Cis-Jasmone	15.9	1380	1380	0.5	0.75	-
31	Germacrene-D	17.4	1453	1450	1.5	3.5	6.5
32	Delta-Cadinene	18.0	1480	1478	-	-	2.2
33	Bicyclogermacrene	18.0	1483	1480	-	1.5	-
34	Spathulenol	19.9	1557	1557	0.4	3.5	5
35	α -Cadinol	21.4	1624	1622	-	-	3.5
36	Muurolol	22.5	1646	1646	-	-	13
Total					97	96	95

Major compounds are shown in **Bold**.
RT: Retention time, a: Kováts retention indices (RI) calculated from retention times in relation to those of a series of C6-C24 n-alkanes on a HP-5MS column.
b: Linear retention indices according to the literature and NIST Chemistry WebBook (-) no detected.

The allelopathy plays major role in plant interactions, invasive plants, diversity conservation, weed management, plant protection against pests and diseases (22). This study aimed to find the best underneath canopy of shrubs (*A. fragrans, T. chiliophyllum,* and *T. polium*) for sowing of *M. scutellata*. Some plant species produce and release diverse and abundant allelochemicals or phytochemicals that inhibit/suppress seed germination and growth of other plants. Medicinal plants have more allelochemicals contents than ordinary plants, hence, these are inhibitory to seeds germination and growth of other plants. These allelochemicals are generally phenolics (such as tannins), alkaloids, steroids, terpenes, saponins, and quinones that adversely affects the growth and development of some neighboring plant species (37). Allelochemicals are classified into 14 categories based on chemical similarity (6). Plant growth regulators (salicylic acid, gibberellic acid and ethylene) are also allelochemicals. Plant essential oil may be considered as potential ecofriendly weed controller due to their phytotoxic effects. According to our results, 1,8-cineol, camphor, α/β-thujone (the main compounds of the essential oils) are inhibitory to *Solanum elaeagnifolium* and *Convolulus arvensis* (31,36). These compounds negatively affects the activity of enzymes (α-amylase and glycolysis), which play an essential role in seeds germination of plants. These compounds also interfere with water uptake by seedlings, gibberellic acid content and mitochondrial activity, thereby affecting the germination.

In addition to the effects of allelopathic compounds on seed germination, these also affects the seedlings growth (radicals and plumule) (1,3). The volatile compounds causes anatomical changes and reduces growth in lettuce, *Bidens pilosa* and *Urospermum picroide* seedlings due to changes in phytohormones, photosynthesis rate, water balance, respiration and protein depletion (1,43). These allelopathic compounds stimulates the plant growth and development by affecting the important physiological processes [cell wall structure, permeability and cell membrane function, inhibition of cell division, activity of some enzymes, plant hormones, seed germination, nutrients uptake, disrupts stomata, photosynthesis, respiration, synthesis of proteins and pigments and alteration of DNA and RNA structure (5,4)].

II. Germination

The applied aqueous extracts of *A. fragrans*, *T. chiliophyllum*, influenced the germination rate, mean germination time and root length of annual *M. scutellata* seeds (Figure 4). The applied aqueous extracts of *A. fragrans and T. polium* inhibited the seeds germination, however, that of *T. chiliophyllum* stimulated the germination. The effects of aqueous extract doses of plant species on various germination indices were dose dependent i.e. with increase in doses, the inhibitory effects increased. Except the Seed vigour Index of *A. fragrans,* Seed vigour Index of both other species was stimulated. In some cases, the aqueous extract (5 %) was not inhibitory to these parameters. *A. fragrans* 20 % aqueous extract was most inhibitory. The allelopathic substances not only affects the seed germination (%) but also affects the emergence of rootlets and plant growth (30). The allopathic potential of plants depends on species type, growth stage and plant organ (21). The highest germination rate (4.61 seeds per day) in control was followed by *T. chiliophyllum* aqueous extract 5 % (2.76 seeds per day). Annual *M. scutellata* seeds germination rate was lowest (1.44 seeds per day) with *A. fragrans* aqueous extract 20 %.

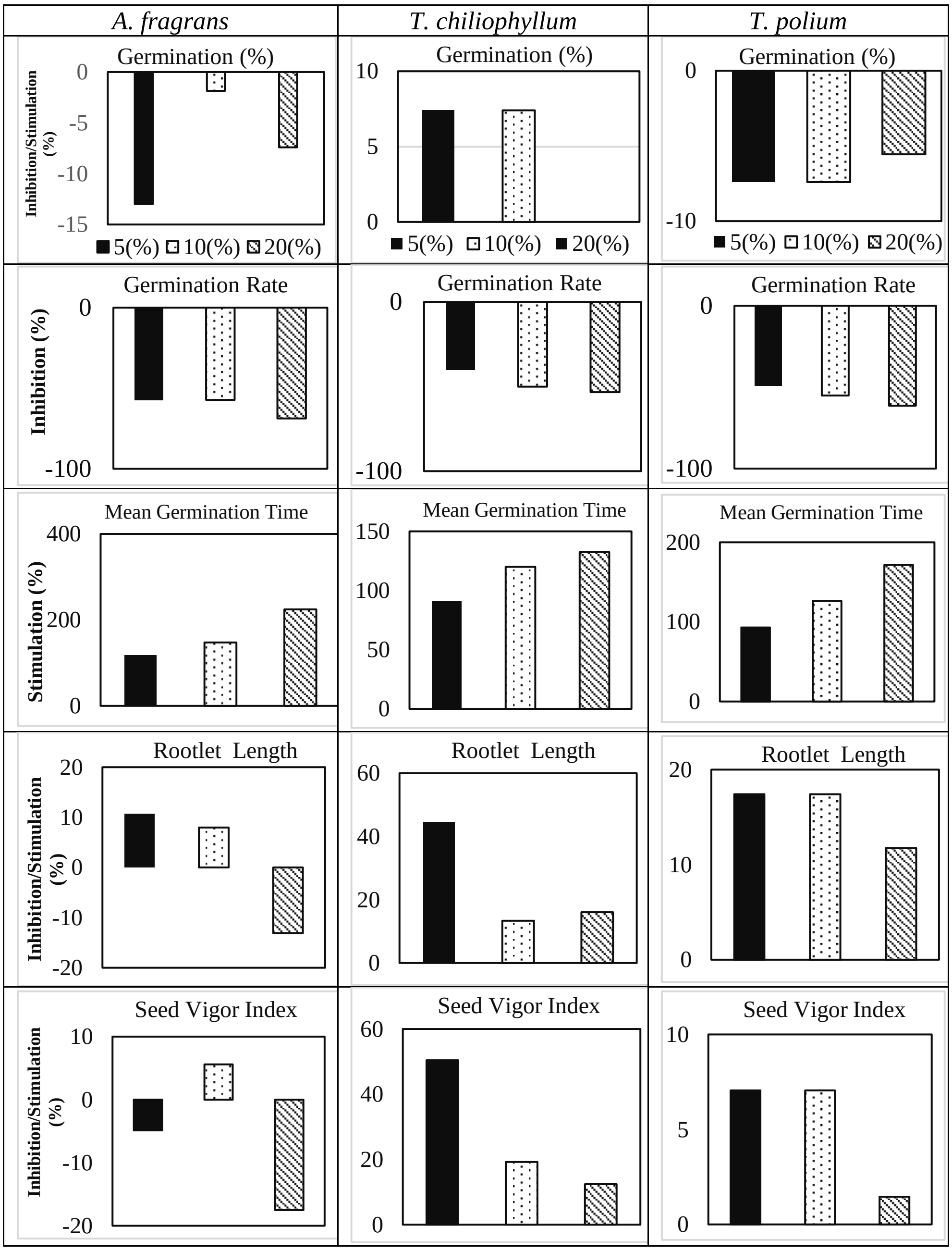

Figure 4. Inhibitory/Stimulatory effects of donor plants aqueous extracts on seed germination of *M. scutellata* at 14-days after sowing.

With increasing concentrations of aqueous extracts of all 3-test species, the germination rate of *M. scutellata* seeds was decreased. Delay in seeds germination is due to changes in the activity of enzymes involved in the transport of storage compounds, which results in shortage of respiratory substrate products and a persistent lack of metabolic energy or ATP. The flavonoids slows the seeds germination via stopping energy transfer (12,47).

On the other hand, the early stages of growth are strongly influenced by seed storage compounds. Seed germination begins with water uptake and is followed by sequential biochemical processes in the seed, which include activation of metabolism, digestion of storage materials and transfer to the embryo, cell division and growth (16). Therefore, slowing of germination in treatments with high concentrations aqueous extracts may be due to changes in the activity of enzymes affecting the transfer of storage compounds during germination (12). The longest mean germination time was in 20 % *A. fragrans* aqueous extract (7.3 days) and shortest in control (2.25 days). The delayed seed germination may be due to the negative osmotic effects of less water absorption and cell elongation (13). The allelopathy inhibits or delays the seeds germination of plants (48).

The *A. fragrans* 20 % aqueous extract inhibited the germination of annual *M. scutellata* seeds. *Artemisia* spp. are known for their allelopathic properties especially against other species that can become invasive in some areas (35). The *A. fragrans* aqueous extracts in all three concentrations were most inhibitory to germination. The *T. chiliophyllum* 5 % aqueous extract were less allopathic to germination of annual *M. scutellata* seeds than other treatments. The allelopathic effects of donor plants on germination indices of *M. scutellata* seeds followed the order: *A. fragrans* > *T. polium* > *T. chiliophyllum.* The increasing aqueous extract concentrations, had negative effects on germination of recipient plants. Seed vigour index in all 3-aqueous extracts of *T. chiliophyllum* was also significantly higher than control.

Allelopathy exists in all plants It has been proposed as a mechanism for influencing the plant populations and communities by influencing the germination and establishment of species. In addition, it is significant ecological factor in determining the structure and composition of plant communities. The inhibitory substances involved in allelopathy are terpenoids and phenolic substances. Sodaeizadeh and Hakimi Maybodi (38) reported the inhibitory effects of *Capparis spinosa*, *Peganum harmala* and *Zygopyllum sp.* aqueous extracts, on alfalfa seed germination. Mohebi *et al*., (24) also reported the inhibitory effects of *Artemisia* spp. aqueous extract of 25 and 75 % concentrations on germination and growth of *Stipa barbata*.

III. Growth

The *A. fragrans*, *T. chiliophyllum*, and *T. polium*, aqueous extracts inhibited the seedlings growth and the inhibition was concentration dependent (Table 5). The *T. chiliophyllum* 5 % aqueous extract stimulated the root length (10.71 cm) than other treatments and control. *T. chiliophyllum* 5 % aqueous extract results were similar to control. Increasing the aqueous extract concentration decreased the growth traits. *A. fragrans* was more inhibitory to measured traits.

The allelopathic responses of *M. scutellata* plant on germination and growth traits to donor plants extracts were similar (Figs 4,5). The increasing concentration of aqueous extracts decreased the plant growth traits.

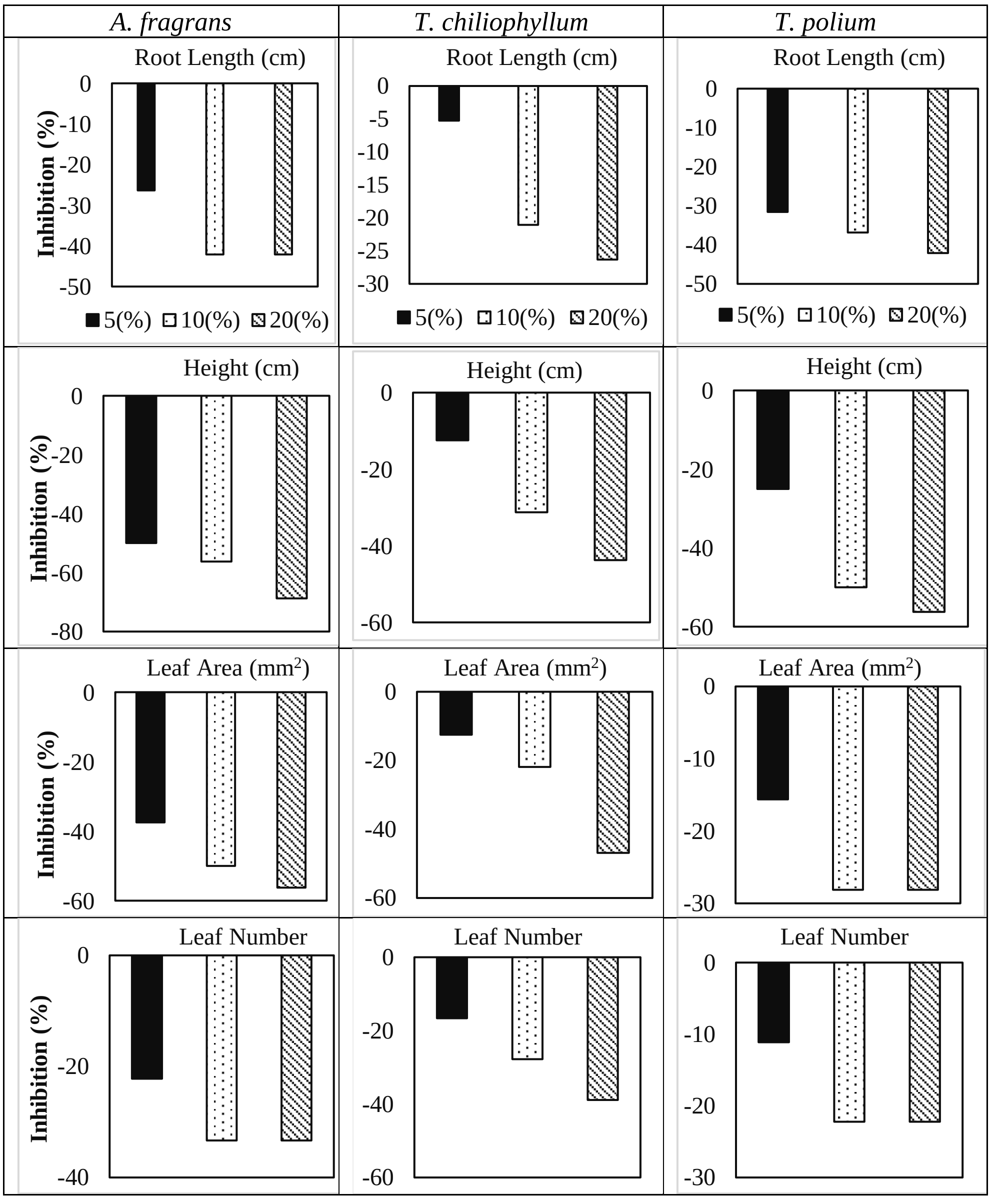

Figure 5. Inhibitory effects of donor plants aqueous extracts on growth of *M. scutellata* at 75 days after sowing.

Based on the results, increase in donor plants density in rangeland (aqueous extract concentration) inhibited the growth of *M. scutellata* plants. The *A. fragrans* had more negative effects on *M. scutellata* establishment. Due to the inhibitory effects of aqueous extract in germination stage, the growth traits are also adversely affected (41). Delayed and slow germination resulted in smaller seedlings and their decreased growth (45).

The addition of allelopathic materials in irrigation water influences the roots morphological and physiological structure (2). In petri plate bioassays, due to direct contact, first uptake and higher allelochemicals concentration around the seedling roots (radicle), the allelopathic effects were stronger on root (radicle) than on shoot (11). In this study, 3-aqueous extracts even at the lowest dose reduced the root growth in seedlings of *M. scutellata*. The higher concentrations of aqueous extracts decreased the growth of leaves, root and shoot length of *M. scutellata* plants. The root lengths affects the plants' growth and health due to their role in nutrients uptakes and providing the physical support to the plant. The association between shorter roots and failure of plants to compete and search for water and minerals from the ground has been reported (39,40). The shorter shoots hinder the plants' ability to develop leaves, which are important for photosynthesis and their less growth may reduce the plant growth (28).

The inhibitory effects on germination and seedling growth of *M. scutellata* followed the order *A. fragrans* > *T. chiliophyllum* > *T. polium*. The *A. fragrans* aqueous extract are inhibitory to seedling growth of 3-alfalfa species (27). If plants are exposed to allelopathy, their growth and development depends on the germination condition. The harmful effects on seed germination are: inhibition or delay in germination, darkening and swelling of seeds, reduced root development, shoots, twisting or curling of the root axis, decreased weight (20,29). Therefore, success of seedings requires good quality of seeds, selection of suitable cultivar, appropriate planting time and sowing depth. Therefore, seed sowing at appropriate depth away from allelopathic material in soil, is necessary to overcome the harmful effects.

Some plants volatile allopathic substances in the environment reduces the seeds germination, but it can be reduced using salicylic acid and gibberellic acid (32), however, more comprehensive research is needed in future. Therefore, it is necessary to pay attention to the allelopathic effects of these 3-species (*A. fragrans*, *T. chiliophyllum* and *T. polium*) on other plants in rangelands. In this regard, if it is necessary to use *M. scutellata* species in sites covered with *A. fragrans*, its seeds may be sown in rainy seasons, when the allopathic substances concentration in *A. fragrans* is lower due to higher soil moisture. On the other hand, based on our results, the areas with moderate relative abundance of *T. chiliophyllum* and even *A. fragrans* did not cause serious problem in the establishment of annual *M. scutellata* species.

CONCLUSIONS

Based on inhibitory allopathic effects, the test donor species were ranked as : *A. fragrans* > *T. polium* > *T. chiliophyllum.* Therefore, the best location to sow seeds of *M. scutellata* is underneath the canopy of *T. chiliophyllum* in rangelands. Regarding the ranking of aqueous extract doses and plant densities, the best site is without shrubs, and in *A. fragrans,* there is no tangible difference of rank among the aqueous extract doses and all three doses had noticeable inhibitory allopathic effects on growth of *M. scutellata*. The essential oils of these plants are rich in terpenoids which are very allelopathic to other plants.

DECLARATION

E. Sheidai-Karkaj and M. Mofidi-Chelan designed and planned experiment. E. Sheidai-Karkaj and A. Alizadeh conducted the experiment. M. Younessi-Hamzekhanlu carried out the chemical analyses of plants. E. Sheidai-Karkaj, M. Mofidi-Chelan and A. Alizadeh wrote article. Z. Baig and S. Aziz contributed in revisions and English editing.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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