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ALLELOPATHIC EFFECT OF *TAGETES MINUTA* L. WATER EXTRACTS ON SEEDS GERMINATION AND SEEDLING ROOT GROWTH OF *ACACIA ASAK*

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ABSTRACT:

Allelopathic effect of water extracts of *Tagetes minuta* (Asteraceae) on seed germination and seedling growth of *Acacia asak* (Mimosaceae) was investigated. The root extracts showed no effect on seed germination percentages while leaf extracts has shown the highest germination inhibition effect. The seedling emergence in sand soil was strongly inhibited by leaf extracts; interestingly, this inhibition of emergence was reduced in sand clay soil. Leaf extracts also had shown the strongest radicle growth inhibition resulted in burning of the radicles' tips. It is concluded that allelochemical exerting the inhibition effect is most abundant in leaves and its effect is reduced with clay sand soil.

INTRODUCTION:

Exotic invasive species are believed to be the second largest cause of biodiversity loss after habitat destruction (Schwartz *et al.*, 1996). It's negative impact on native biodiversity has been well documented (D'Antonio and Mahall 1991, Ridenour and Callaway 2001). Exotic invasive plants have been found to change the composition of native communities and been associated with reduced plant and insect diversity (Bone *et al.*, 1997, Ferrcia and Marques1998). It can also affect soil microbial and arthropod composition (Batten, *et al.* 2006, Pritekel *et al.*, 2006). Exotic invasive species threatening biodiversity and habitat quality by displacing native plants; forming dense stands that exclude natives; hybridizing with natives thereby changing their genetics and supporting other non-native organisms.

Most of exotic plants effects reported have been identified as caused by allelopathic interaction resulted in interference with physiological and biochemical processes in plant due to chemicals released by exotic plant. However, previous investigations of the allelopathic effects of exotic plants have been concerned with its impact on agricultural crops (Khalid *et al.*, 2002). It is important to assess the impact of these plants on non-agricultural plants such as *Acacia asak* (Mimosaceae) which represent one important source of fuel, traditional agricultural tools, and feed for goats, camal, and bees.. In Yemen, the forest, rangeland and roadsides where *Acacia asak*

usually grow are highly infested with *Tagetes minuta* as an exotic invasive plant of South American origin. The causes of reduction in numbers of *Acacia asak* plants are may be due to the impact of *Tagetes minuta*. The objectives of this study are to assess the allelopathic effect of *Tagetes minuta* extracts on germination inhibition of *Acacia asak* seeds and seedling radicle growth reduction, and to determine the *Tagetes minuta* plant organs which contains the allelochemicals that cause inhibition of seeds germination and root growth.

MATERIAL AND METHODS:

Seeds of *Acacia asak* (Forssk.) Willd. (Mimosaceae) were collected from Almawaseet district, Taiz governorate, Yemen. Seeds were air dried and stored in plastic bags at room temperature till use. *Tagetes minuta* L. (Asteraceae) plants were collected from Sana'a university new campus, as mature flowering plants including roots. It was immediately transferred to the laboratory where large mature leaves were collected in addition to stems and roots. The three plant's organs were cut into small pieces. The pieces of each organ where socked in a distilled water for 24 hours at room temperature and for other 24 hours in refrigerator to avoid fermentation and continue the extraction process. The amount of water was three times of the plant organs (V/W). The extracts were filtered through filter papers and stored in refrigerator till use. The germination assays were performed by placing seeds (three replicates of 50 seeds each) in 20 cm diameter petri dishes containing filter paper moistured with distilled water (control), leaf, stems, and roots water extracts at room temperature 25±3◦C. Seeds were considered to have germinated when radicle had emerged. For seedling emergence assay same day distilled water germinated seeds were transferred to trays containing sand and sand clay soil and were watered with distilled water, leaves, stems, and roots water extracts. This step was meant to assure similar age to all seedlings and to eliminate other possible causes of seedling emergence prevention like delayed germination. Then percentages of seedling emergence were calculated. In the same way root length assay was done. Data analysis was done using Minitab statistical package version 12. Arcsine transformation of data was made for percentages to meet the ANOVA assumptions.

RESULTS AND DISCUSSION:

The percentage of germinating *Acacia asak* seeds were germinated in distilled water (control) leaf, stem, and root extracts of *Tagetes minuta* indicated that the root extract is showing no effect on germination of *Acacia asak* seeds whereas leaf extracts exerted the strongest inhibitory effect followed by stem (Fig.1). This result clearly show the allelopathic effect of *Tagetes minuta* leaf extracts on *Acacia asak* seed germination. It is also show the organs of *Tagetes minuta* plant accumulate the allelochemical is the leaves.

The seedling emergence percentages of *Acacia asak* in sand soil irrigated with leaf, stem, and root extracts of *Tagetes minuta* were 6%, 82%, and 89%, for leaf, stem and root extracts respectively compared to 99% in distilled water (Fig. 2). Even the low percentage of germinated *Acaia asak* seeds will face another stage challenge that is the emergence of seedlings which is retarded by leaf extracts of the *Tagetes minuta* plants. The aqueous extracts are specifically mimicking what is taking place in nature instead of using chemical extraction process. This can explain field observation which has shown dominance of *Tagetes minuta*

in most waste land and roadsides resulted in prevention of seed germination and growth of other plants.

Interestingly, the emergence percentage of seedlings in sandy clay soil has shown 33%, 26%, and 30% for leaf, stem, root extracts respectively and 70% in control (distilled water) (Fig. 3). However, the presence of some clay soil is resulted in increasing the emergence percentage of *Acaia asak* seedlings compare to pure sand soil. To show clearly the role of clay in reducing the leaf extract effect well designed field experiment is recommended. The clay particles or the chemical composition of the soil or may be clay microbial life may play a role in reducing the impact of *Tagetes* leaves allelochemical. While three days old seedlings' radicle has not shown statistically significant reduction as a result of *Tagetes minuta* plant extracts treatments (Fig. 4 A& B). Interestingly, the root length of *Acacia asak* seedling grown for seven days in sand soil was negatively affected by leaf extracts followed by stem extracts. *Acacia asak* root tips burning was seen as result of leaf extract effect on the seedling root growth (Fig. 5). Similarly the shoot length of *Acacia asak* seedlings was also greatly reduced by *Tagetes minuta* leaf extract, the stem has shown moderate reduction while the root extracts has not shown any influence (Fig. 5). Singh *et al.* (2006) concluded that α-pinene inhibits early root growth and causes oxidative damage in root tissue through enhanced generation of reactive oxygen species (ROS), as indicated by increased lipid peroxidation, disruption of membrane integrity and elevated antioxidant enzyme levels. They found α-pinene inhibited the radicle growth of all the tested plant species. Exposure of roots to α-pinene (allelochemical) enhanced solute leakage, and increased levels of malondialdehyde, proline and hydrogen peroxide, indicating lipid peroxidation and induction of oxidative stress. Similar mechanism can explain the burning of roots tips of *Acacia asak* by leaves extracts of *Tagetes minuta* and inhibition of germination. This burning can may be explained by toxicity. Oracz *et al.* (2007) demonstrated that despite the activation of the antioxidant system by sunflower phytotoxins, reactive oxygen species accumulation caused cellular damage, which resulted in the decrease of germinability. They concluded that, the negative effect of sunflower on germination of mustard seeds is mostly because of its toxicity and not to its contribution to osmotic potential.

The impact of invasive plant is not only restricted to these effects. Recently Stinson *et al.* (2006) presented evidence which elucidate an indirect mechanism by which invasive plants can impact native flora. They found that antifungal phytochemistry of the invasive plant, *Alliaria petiolata*, suppresses native plant growth by disrupting mutualistic associations between native canopy tree seedlings and belowground arbuscular mycorrhizal fungi. These result in addition to Topp *et al.*, (1998) finding on which they conclude that *Tagetes* do not cause a general depression in the numbers of microorganisms in soils, and that nematode control by *Tagetes* may not be due to the release of a biocidal agent into the soil necessite the need to investigate the mechanism of *Tagetes* allelopathic effect.

Isolation of allelochemical and investigation of mode of action is recommended. The allelochemicals of *Tagetes* can be used as herbicides. *Tagetes minuta* is allelopathic to *Acacia asak* it is might be the main cause of population reduction.

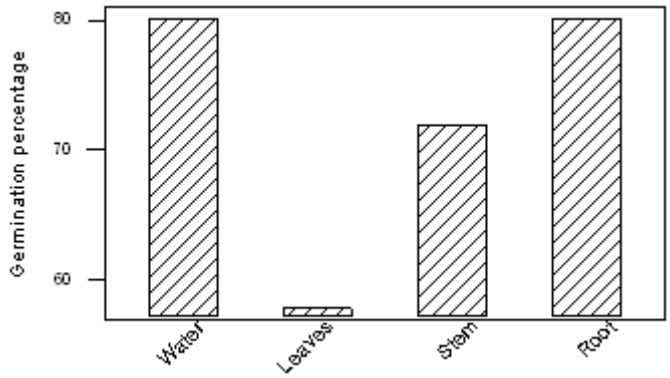

Fig. (1): Germination percentage of *Acacia asak* seeds in water, leaf, stem, and root extracts of *Tagetes minuta* plant

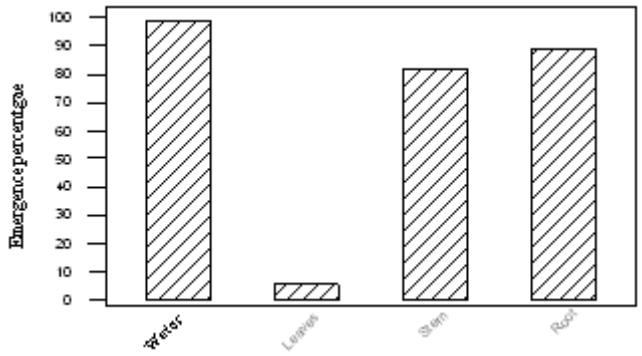

Fig. (2): Emergence percentage of *Acacia asak* seedlings planted in sandy soil after germination in water and irrigating it with water, leaf, stem, and root extracts of *Tagetes minuta* plant

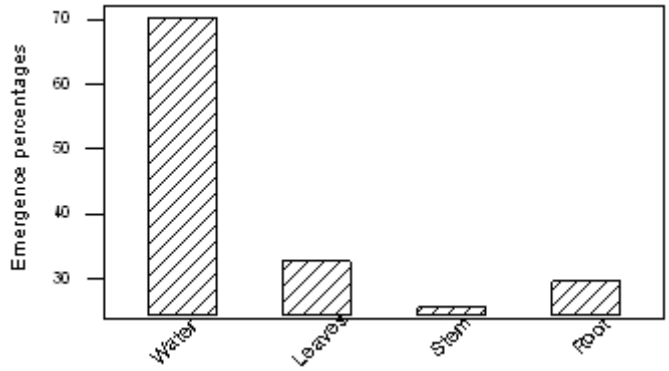

Fig. (3): Emergence percentage of *Acacia asak* seedlings planted in sandy clay soil after germination in water and irrigating it with water, stem, and root extracts of *Tagetes minuta* plant

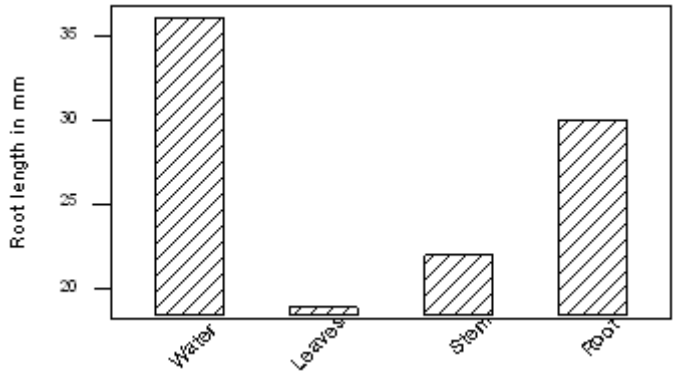

Fig. (4 A): Radicle length of *Acacia asak* seedlings three days after germination in water, planted in sand soil and irrigated with water, leaf, stem, and root extracts of *Tagetes minuta* plant

Fig. (4 B): Radicle length of *Acacia asak* seedlings three days after germination in water, planted in sand soil and irrigated with water, and leaf, stem, and root extracts of *Tagetes minuta* plant

Fig. (5): Radicle and shoot length of *Acacia asak* seedlings germinated in water, planted in sand and irrigated with water, and leaf, stem, and root water extracts of *Tagetes minuta* plant Note the burning of root tip in seedling germinated in leaf extracts

Annex (1)

One-way ANOVA showing the effect of different *Tagetes minuta* plant parts extracts and water on;% of germination of *Acacia asak* seeds, emergence% of *Acacia asak* seedlings in sand soil, emergence% of *Acacia asak* seedlings in sand clay soil, Root length of *Acacia asak* seedling three days after germination and Root length of *Acacia asak* seedling seven days after germination. Arcsine transformation was made for%s to meet the ANOVA assumptions.

Analysis of Variance for germination

Source	DF	SS	MS	F	P
Treatment	3	367.78	122.59	14.60	0.001
Error	8	67.18	8.40		
Total	11	434.97			

Analysis of Variance for emergence in sand soil

Source	DF	SS	MS	F	P
Treatment	3	8663.1	2887.7	202.76	0.00
Error	8	113.9	14.2		
Total	11	8777.0			

Analysis of Variance for emergence in sand clay soil

Source	DF	SS	MS	F	P
Treatment	3	1319.2	439.7	35.68	0.00
Error	8	98.6	12.3		
Total	11	1417.8			

Analysis of Variance for root length 3 days after germination

Source	DF	SS	MS	F	P
Treatment	3	116.3	38.8	35.68	0.217
Error	8	168.0	21.0		
Total	11	284.3			

Analysis of Variance for root length seven days after germination

Source	DF	SS	MS	F	P
Treatment	3	5986.0	1995.3	155.48	0.000
Error	8	102.7	12.8		
Total	11	6088.7			

REFERENCES:

Batten, K. M., Kate M. Scow, Kendi F. Davies 3 & Susan P. Harrison (2006): Two invasive plants alter soil microbial community composition in serpentine grasslands Biological Invasions 8:217–230.

Bone, R., Lawrence M, Magombo, Z. (1997): The effect of a *Eucalyptus camaldulensis* (Dehn) plantation on native woodland recovery on Ulumba Mountain, southern Malawi. Forest ecology and management 99: 83-99

D'Antonio, C.M. and Mahall, B.E. (1991): Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. *Am. J. Bot.* 78, 885–894

Ferreira, R. L. and Marques, M. M. (1998): Litter fauna of arthropods of areas with monoculture of *Eucalyptus* and heterogeneous secondary forest. Anais da Sociedade Entomológica do Brasil. 27: 395-403

Khalid, S, T. Ahmad and R. A. Shad (2002): Use of Allelopathy in Agriculture. Asian Journal of Plant Sciences 1: 292-297.

Oracz K., Christophe Bailly, Agnieszka Gniazdowska, Daniel Côme, Francoise Corbineau and Renata Bogatek (2007): Induction of Oxidative Stress by Sunflower Phytotoxins in Germinating Mustard Seeds. Journal of Chemical Ecology 33: 251–264.

Pritekel, C; Whittemore-Olson, A and Snow, N; Moore, JC. (2006): Impacts from invasive plant species and their control on the plant community and belowground ecosystem at Rocky Mountain National Park, USA. Applied Soil Ecology. 32:132-141.

Ridenour, W.M. and Callaway, R. M. (2001): The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126, 444–450.

Schwartz M. W., Daniel J. Porter, John M. Randall and Kelly E. Lyons (1996): Impact of Nonindigenous plants in Sierra Nevada Ecosystem Project: Final report to Congress, Vol. II, Assessments and scientific basis for management options. Davis: University of California, Centers for Water and Wildland Resources, 1996. www.ceres.ca.gov/snep/pubs/web/PDF/VII_C47.PDF (downloaded 18/2/2003).

Singh H P., Daizy R. batish,, Shalinder Kaur, Komal Arora and Ravinder K. Kohli (2006): α-Pinene Inhibits Growth and Induces Oxidative Stress in Roots Annals of Botany 98: 1261–1269, 2006

Stinson K. A., Stuart A. Campbell, Jeff R. Powell, Benjamin E. Wolfe, Ragan M. Callaway, Giles C. Thelen, Steven G. Hallett, Daniel Prati, John N. Klironomos (2006): Invasive Plant Suppresses the Growth of Native Tree Seedlings by Disrupting Belowground Mutualisms. PLoS Biology 4: 727-731

Topp E., S. Millar, H. Bork, and M. Welsh (1998): Effects of marigold (*Tagetes* sp.) roots on soil microorganisms. Biology and Fertility of Soils 27: 149-154.

التأثير الاليلوباثي لمستخلصات نبات ***Tagetes minuta*** (الفصيلة النجمية) على إنبات ونمو جذور العسق ***Acacia asak*** (الفصيلة الطلحية)

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تم دراسة التأثير الاليلوباثي للمستخلصات المائية لنبات القطيفة البري من الفصيلة النجمية على إنبات ونمو جذور نبات العسق من الفصيلة الطلحية، ولم يظهر مستخلص الجذور أي تأثير على نسبة الإنبات، فيما أظهر مستخلص الأوراق تأثير مثبط قوي للإنبات ولبزوغ بادرات العسق في التربة الرملية، وأدى وجود التربة الطينية إلى تخفيف التأثير المثبط لمستخلص الأوراق على بزوغ بادرات العسق. وأدى مستخلص الورقة المائي للقطيفة البرية إلى تثبيط نمو جذور العسق وحرق قممها. نتسنتج من هذه الدراسة أن المادة الكيميائية المثبطة أكثر تواجداً في أوراق نبات القطيفة البري، ويقل تأثيرها في التربة الطينية مقارنة بالتربة الرملية.