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Canopy effect of *Prosopis juliflora* on selected grass species by using soil aqueous extract in Afar Regional State, Ethiopia

Ali Noha, Lisanework Nigatu, Rejila Manikandan*

School of Natural Resources Management and Environmental Sciences, College of Agriculture and Environmental Sciences, Haramaya University, Haramaya, Dire Dawa, Ethiopia

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Abstract

Allelopathy effect of *Prosopis juliflora* canopy soil aqueous extracts on tropical grass species such as *Cenchrus cillaris, Paspalidium desertorum* and *Lintonia nutans* were tested under in vitro conditions. Leaf litter falling under canopy soil was collected and tested for allelopathic effects of soil aqueous extract under natural conditions. All the soil aqueous extracts showed significantly negative effects on both germination and seedling growth of test grass species. The results showed that seed germination percentage decreased with increasing the level of the concentration as well as canopy distance. In *Cenchrus ciliaris* grass species showed 41.64 and 28.56% reduction in seed germination with 5 and 10% soil aqueous extract respectively, in 0 to 3 meter canopy distance. Whereas, at the same concentration level, the seed germination percentage was 57 and 53% in 3 to 10 meter canopy distance. A similar trend of reduction in seed germination percentage was also noticed in *Paspalidium desertorum* and *Lintonia nutans* grass species. Moreover, this study revealed that seedling growth such as shoot and root length was significantly affected by canopy soil extracts at the highest concentration. The effect of 0 to 3 meter canopy soil extract was the highest, followed by 3 to 10 meter canopy soil extracts. Moreover, at low concentration canopy, soil aqueous extract showed less inhibitory effect as compared with higher concentration. It can conclude that *Prosopis juliflora* contains water-soluble allelochemicals capable of inhibiting selected grass species and *Prosopis* is not good for agro ecosystem and biodiversity.

* **Corresponding Author:** Rejila Manikandan ✉ rejularaja@gmail.com

Introduction

Prosopis juliflora is native to Central and South America and then started to spread from southern Mexico to Panama and from the Caribbean Islands to Venezuela and northern Peru (Bokrezion, 2008). Nowadays, it can be found in various semi-arid and arid climate zones, including Ethiopia. It was first introduced to the Awash area of the Afar Regional State some 30 years ago (Zaraye, 2008). *Prosopis juliflora* competes with grasses and indigenous trees and has invaded farmland and rangelands in the area. The farmland causes great damage to biodiversity, livestock and food production. *Prosopis* has a wide adaptability of soil ranging from sand dunes to clay soils, from saline to alkaline soils, from areas below 200 to more than 1500 m above sea level and mean annual rain fall ranges between 50 to 1500 mm. It can also withstand and survive temperatures from as high as 50°C (air temperature) and 70°C (soil temperature). It is one of the most common trees in semi-arid and arid parts of the sub-tropical and tropical zones (Pasieczik *et al*., 2004).

In Ethiopia, *Prosopis juliflora* is considered a major threat because of its invasive nature. It has an aggressive invasive character, invading pastureland, irrigated cultivated lands and irrigation canals, causing an irreversible displacement of natural pasture grasses as well as native tree species. In terms of *Prosopis juliflora* coverage, the area's most adversely affected nationally include the Afar and Somali Regions in the east and southeast of the country and the area around Dire Dawa City. There are also moderately affected areas in Amhara, Oromia, Southern Nations Nationalities and Peoples (SNNP) and Tigray Regions, that is, in the mainly dry lands of Central, East and North Ethiopia (Steele *et al*., 2009).

Prosopis juliflora is one of the invasive alien plant species that are threatening the native plant species. It grows in very hot, dry climates, with a temperature up to 48°c and annual precipitation from 150 to 750 mm. *Prosopis* root reaches to great depth in the soil searching for the required water. It can grow in a variety of soil types, including saline and alkaline areas in sandy and rocky soils.

The tissue of *Prosopis juliflora* is photo synthetically active throughout the year, presenting a wide spread root system through which the tree fully exploits the available water resources. Its low nutritional requirements and resistance to water deficit give *Prosopis juliflora* a great plasticity of response, which allows its wide distribution in arid and semiarid zones in the tropics (Pasiezik *et al*., 2001). However, the *Prosopis* decline in growth of the annual grasses like *A. adscensionis* during invasion was most likely due to competition for light (shading) from invading *Prosopis juliflora*, the increases in the annual grasses *C. virgata* and *S. verticillata*, the perennial grass *C. dactylon* and the non-succulent shrubs *A. lindleyi* and *S. tuberculata* were most probably in response to the establishment of favorable environmental conditions in the sub canopies and neighborhoods of the trees. *Aristida adscensionis* performs poorly in shade while *C. virgata, C. dactylon* and the alien non-succulent shrub *A. lindleyi* tolerate shade and are hardy colonizers of bare or disturbed soil (Nidhlovu *et al*., 2016).

Allelopathy is one of the interesting properties found in some plants as *Prosopis species* with a depressive effect on the associated flora attributed to the release of chemicals that affect other plants. Moreover, the negative impact of the plant could be through light deprivation, competition for water and nutrients, or leaching of Allelopathic compounds. Some of the plant secondary metabolites are called allelochemicals or their products that are released into the environment and may affect neighboring organisms by affecting their physiological activities. These species are causing enormous damage to biodiversity as well as agricultural systems (Bais *et al*., 2003). Therefore, this research was initiated to study the canopy effect of *Prosopis juliflora* on selected grass species through soil aqueous extract.

Materials and methods

Collection of Soil Sample for Soil Bioassay

Soil at a depth from a range of 0-3cm to 3-10cm and distances between 0-3 meters and 3-10 meters were collected separately under several mature stands of *Prosopis juliflora* canopy. Soil was also collected at the distance of 25 meters outside of the *Prosopis juliflora* canopy to serve as a control.

The composite soil samples from different distances were separately air dried and sieved through a 5 mm sieve to remove large clods of dirt, roots and other vegetative materials. After this, soil samples were kept in a refrigerator for further soil bioassay study.

Collection of grass Species

The three most important and predominant pastoral types of grass before the *Prosopis* invasion in the study area were purposely selected and the seeds of grasses were collected from the Gene Banks of Werer Agricultural Research Centre. Seeds of three grass species, such as, *Paspalidium desertorum, Cenchrus ciliaris* and *Lintonia nutans*.

Preparation soil aqueous extract

Aqueous extracts were prepared using the extraction procedures of (Haisey, 1990). Ten grams of the soil was dissolved separately in 100ml of distilled water in conical flasks and shaken for four hours. Following extraction, the solution was filtered through double layers of cheese cloth and filter paper, the volume was made up to 100ml of distilled water and kept at 40C until application. The aqueous extract of the soil was prepared at 0%, 1%, 2.5%, 5% and 10% concentrations (Lisanework and Michelsen, 1993). The filter paper was moistened with 5ml of the aqueous extracts of 1%, 2.5%, 5%, and 10% and distilled water in case of control. In a Petri-dish, twenty-five selected grass species seeds were spread out on two sheets of filter paper. There were three replicated Petri-dishes for each type of extract concentration and recipient species. The Petri-dishes were randomized and incubated at 200C in darkness in sealed plastic bags. The germination was recorded 96hrs after onset of germination whereas plumule and radical length were measured after seven days of its onset respectively.

Soil bioassay study on selected grass species

Seeds of three grass species: *Cenchrus cillaris* L. (white), *Paspalidium desertorum* and *Litonia nutans* have been germinated following the methods. The filter paper was moistened with 5ml of under canopy soil aqueous extracts of 1% 2.5%, 5%, and 10% and distilled water in case of the control.

In Petri-dishes, twenty-five seeds were spread out on two sheets of filter paper. There were three replicate Petri-dishes for each type of extract concentration and recipient species. The Petri-dishes have been randomized and incubated at 20°C in darkness in sealed plastic bags. Germination percentage, plumule and radical length were measured 96hrs (48 hrs) after the onset of germination. Germination percentages were calculated after the onset of germination.

$$\text{Germination (\%)} = \frac{Number of seeds germinated}{Number of seeds placed for germination} x100$$

Data analysis

The data collected on germination bioassay and greenhouse experiment were subjected to analysis of variance using SAS (version 9) software program. Simple descriptive statistics, one way ANOVA and Duncan's multiple range tests were used for data analysis. All data were tested at $p<0.05$ level in order to investigate if significant differences existed among treatments.

Results

Impacts of Prosopis juliflora on seed germination percentage of selected grass species

Effect of *Prosopis juliflora* canopy soil aqueous extracts on seed germination percentage of selected grass species such as *Cenchrus ciliaris*, *Paspalidium desertorum* and *Lintonia nutans* were analyzed and the results were summarized in table-1. The results showed that, seed germination percentage was decreased with increasing the level of the concentration as well as canopy distance. In *Cenchrus ciliaris grass* species showed41.64 and 28.56% reduction in seed germination with 5 and 10% soil aqueous extract respectively in 0 to 3 meter canopy distance. Whereas, at the same concentration level seed germination percentage was 57 and 53% in 3 to 10 meter canopy distance. Similar trend of reduction in seed germination percentage also noticed in *Paspalidium desertorum and Lintonia nutans* grass species. In this experimental analysis, among the selected grass species *Lintonia nutans*is highly affected the seed germination in 5 and 10% soil aqueous extract and also inhibitory effect was more in close canopy.

However, delayed germination of seed germination under allelopathic stress might be due to imposition of dormancy on seeds by allelochemicals and seeds might germinate whenever conditions turn favorable due to loss of allelopathic substances from soil (Narwal, 1994).

Table 1. *Prosopis juliflora* canopy effect (soil aqueous extract) on seed germination percentage of selected grass species.

Soil Bioassay Concentration	*Cenchrus cillaris*		*Litonia nutans*		*Paspalidium desertorum*	
	0-3 meters distance	3-10 meters distance	0-3 meters distance	3-10 meters distance	0-3 meters distance	3-10 meters distance
0	98.56%	98.56%	82.35%	82.35%	97.0%	97.0%
1%	60.72%	71.20%	45.36%	69.00%	69.00%	74.25%
2.5%	49.20%	57.00%	33.84%	49.44%	65.00%	69.80%
5%	41.64%	53.00%	32.25%	42.24%	57.40%	64.84%
10%	28.56%	42.16%	29.12%	34.76%	50.60%	54. 65%

Prosopis juliflora canopy effect (soil aqueous extract) on seedling growth of selected grass species under *in vitro* condition

Effect on Shoot Growth

Aqueous extracts of under canopy soil of *Prosopis juliflora* were exhibited significant effects on the shoot length of *Cenchrus cillaris* at all concentration levels but extremely suppressed at 5 and 10% concentration. At the lowest concentration the inhibition on shoot growth was not significant as compared with control plant.

Regarding, the canopy distance 0 to 3meters distance causes more inhibition on shoot growth of *Cenchrus cillaris* as compared with 3 to 10 meters distance. This variation may be due to the accumulation of more allelochemicals in close canopy proximity. Moreover, when the distance increase from the *Prosopis* canopy decrease the concentration of allelochemicals

Litonia nutans showed the shoot growth ranges from3.8 to 2.0 for 0 to 3 meters canopy distance. Where as in 3.8 to 2.6 cm growth showed for 3 to 10 meters canopy distance. However, the inhibitory effect was observed in all the concentration as compared with control group.

The more inhibitory effect was observed at the highest level of concentration. Similar trend of inhibitory effect was also noticed in *Paspalidium desertorum* grass species.

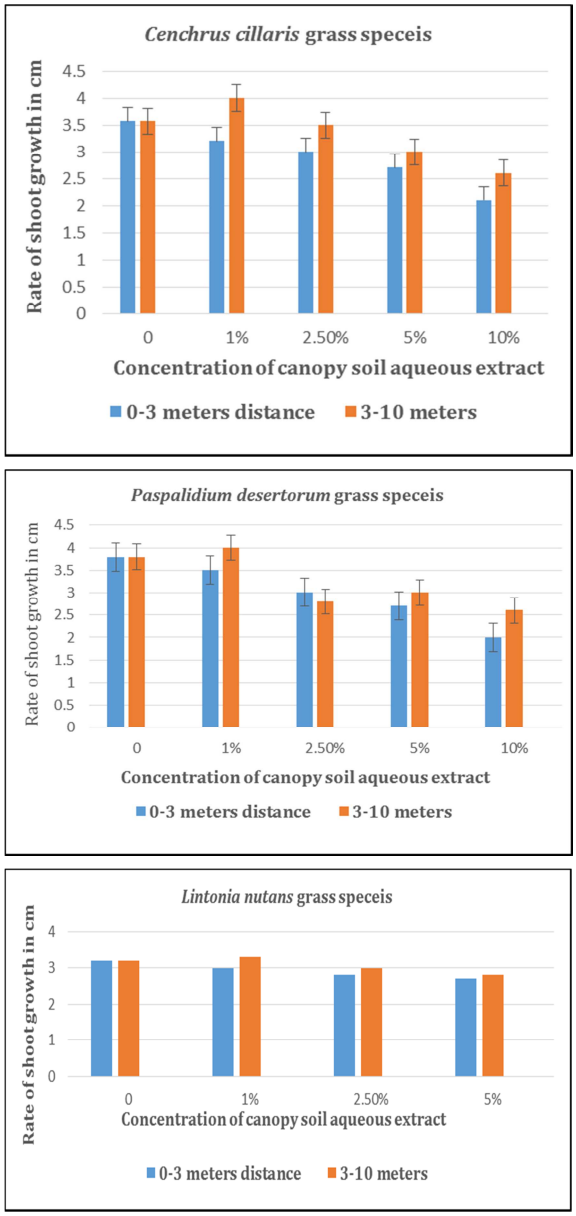

Fig. 1. *Prosopis juliflora* canopy effect (soil aqueous extract) on shoot growth of selected grass species under in vitro condition.

Effect on Root Growth

The average root length (cm) recorded from the germinated seedlings 15 days after planting are presented in figure-2. The allelopathic effects of canopy soil aqueous extract on root length of the test grass species was demonstrated as that root length of was significantly affected upon applying the different concentrations of extracts. According to the results recorded the highest extract concentrations of had significant effect on shoot and root length of *Cenchrus cillaris* when compared with the control, root lengths of the seedlings were significantly ($P<0.05$) reduced by canopy distance at all concentration levels.

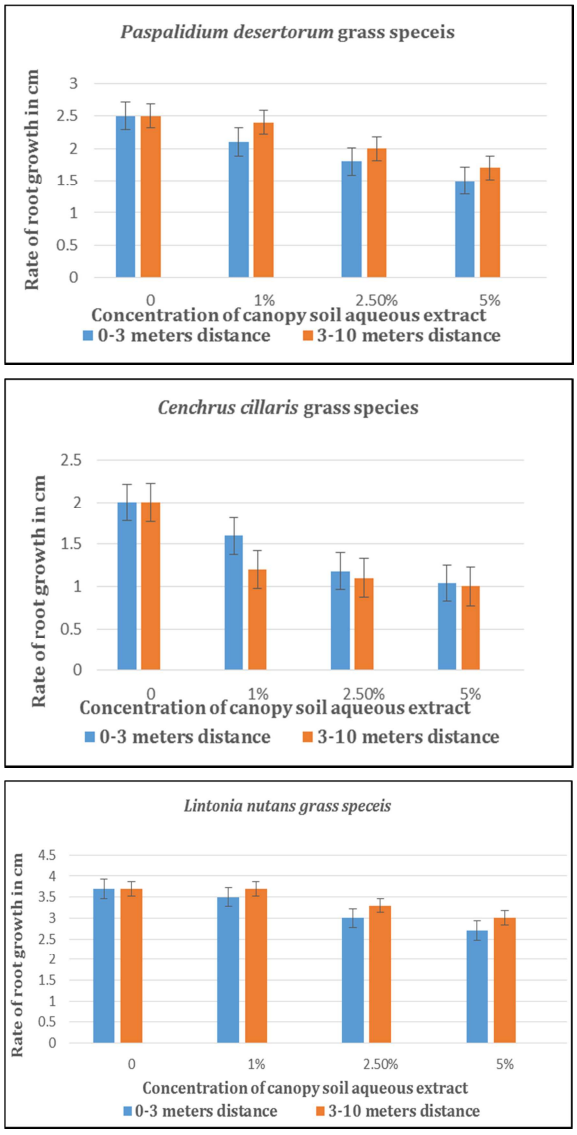

Fig. 2. *Prosopis juliflora* canopy effect (soil aqueous extract) on root growth of selected grass species under *in vitro* condition.

Reduction in growth increased with increasing extract concentration. However, the maximum reduction of the root length was observed at 5% soil aqueous extract as compared to all the treatments. *Paspalidium desertorum* showed the shoot growth ranges from 2.5 to 1.5 for 0 to 3 meters canopy distance. Where as in 2.5 to 1.7 cm growth showed for 3 to 10 meters canopy distance. However, inhibitory effect was observed in all the concentration as compared with control group. The more inhibitory effect was observed at the highest level of concentration. Similar trend of inhibitory effect was also noticed in *Litonia nutans* grass species.

Discussion

The impact of canopy soil aqueous extract on seed germination of selected grass species was concentration dependent as well as canopy distance. In line with this, Verma and Rao (2006) reported that the inhibitory effect of seed germination depends on the concentration of allelochemicals accumulated on the canopy soil surface. It is also suggested by some researchers that the presence of toxic compounds on some plant liters under the canopy of the tree species (Setia *et al.*, 2007). Hossian and Alam (2010) reported the allelopathic property and inhibit the seed germination of other plants due to the presence of secondary compounds such as phenolic acids and some other phytotoxic chemicals released from the decomposition of leaf litter and roots. Leaf extracts of invasive weed species have also been reported to negatively affect germination of agricultural crops such as *Triticum aestivum* and *Cucurbita pepo* (Hossian and Alam, 2010).

However, 0 to 3 meters showed more inhibition than 3 to 10 meters. This may suggest that *Prosopis juliflora* contains a significant source of water soluble allelochemical growth inhibitors. This result clearly showed that *P. juliflora* has an allelopathic effect on the seed germination percentage of selected grass species, which is in agreement with the reports of Dongre and Sing (2007) who reported that *P. juliflora* significantly inhibited seed germination in Pearl millet. Such inhibitory effects of *P. juliflora* have also been reported by Sundaramoorthy *et al.* (2004)

aqueous leaf extracts of *P. juliflora* showed inhibitory effects on germination of *Cynodondactylon*. Similarly, Al-Humaid and Warrage (1998) have found that aqueous extract application of *P. juliflora* significantly reduced the germination and seedling growth of wheat. They also indicated that the maximum degree of inhibition occurred when leaf extract was used rather than stem and root extracts.

The results of the present finding signify that canopy soil aqueous extracts at all concentration levels were significantly (P< 0.5) reduced shoot and root lengths when compared with the control. Growth was also found to progressively decrease with increasing extract concentrations as well as canopy distance. Aqueous extracts of *P. juliflora* were also reported to reduce seedling growth in *B. campestris*. And inhibited root and shoot growth of *Cynodon dactylon* (Sundaramoorthy *et al.*, 2004). Moreover, *Prosopis* extract causes the maximum reduction in root length of wheat reported by Khan *et al.* (2004). Siddiqui *et al.* (2009) indicated the growth inhibitory effect of syringin and lariciresinol isolated from *P. juliflora* on lettuce seedlings and Barnyard grass (Nakano *et al.*, 2001)

Conclusion

The current research specifies that the presence of water soluble and biodegradable phytotoxic substances of *Prosopis juliflora* are capable of preventing seed germination and seedling growth of selected grass species such as *Cenchrus cillaris, Paspalidium desertorum* and *Lintonia nutans*. The secondary compounds found in leaves, litter and root extracts as well as the under canopy soil have enough concentration of phototoxic compounds to affect the nearby plants. Hence, it is capable of reducing germination, and significantly inhibiting seedling growth of grass species. For the open canopy of *Prosopis juliflora* light prevention cannot explain why grasses are eliminated after the introduction of the species. Invaded by allopathic plants such as *Prosopis juliflora* and its exudates could be one of the reason disappearances of native grass species in the agroecosystem. Thus, prevention of *Prosopis juliflora* encroachment on to grass land and protecting the native grass land species.

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