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Effects of leaf extracts of *Lantana camara* and soils invaded by it on *Lepidium sativum* growth performance

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Abstract
Lantana camara is a noxious invasive plant that invades agricultural and natural ecosystems. In the current study, phytotoxicity of hexane and ethanolic leaf extracts of *L. camara* in different concentrations, and soils invaded by it on *Lepidium sativum* was investigated under laboratory conditions. Soil toxicity was evaluated by comparing growth of *L. sativum* on soils sampled from lantana invaded and lantana-free sites. Results showed that extract concentrations and solvent type, and their interaction significantly reduced percent seed germination and seedling growth. Compared to control (distilled water), both hexane and ethanol extracts at 5% w/v concentration significantly reduced percent germination and early seedling growth, and completely inhibited seed germination at 10% w/v of hexane leaf extract, suggesting that hexane extract has greater inhibitory effect than ethanolic extract in all the parameters measured. However, growth performance and seed yield of *L. sativum* grown on soil invaded by lantana did not significantly vary from those grown on soils sampled from non-invaded sites. The results of this study generally showed that though lantana leaf extracts have direct negative allelopathic effect on *L. sativum*, soils invaded by lantana have no toxic materials in the soil to directly or indirectly inhibit growth of *L. sativum*. Further field studies on allelopathic effects of lantana on *L. sativum* are recommended.

Keywords: allelopathy, invasive plants, seed germination, seedling growth, soil quality

Introduction
Allelopathy, an interesting phenomenon, encompasses retardation or stimulation of growth of one plant by dominance of other. Allelopathic potential of certain plants cause detrimental impact on germination and growth parameters of crops and weeds by allelochemicals released by the them (Rice, 1974; Sahid and Sugau, 1993)[1,2]. Allelochemicals that are released into the environment through leaching, litter decomposition, root exudation, or direct volatilization may inhibit seed germination, seedling growth and establishment and yield of neighboring crop (Oudhia, 2000) [3]. They may exert negative impacts through modification of resource consumption capacity; alteration of cell membrane permeability and enzymatic activity; triggering genetic defects and disturbing photosynthesis of the recipient plants (Majeed *et al*., 2012) [4].
Lantana camara L. (Verbenaceae) is among the top ten invasive plant species (Sharma *et al.*, 2005) [5] growing in many countries under a wide range of climatic conditions and soil types (Binggeli and Dessissa, 2002; Day *et al*., 2003; Zalucki *et al*., 2007) [6-8]. Because of its invasive aptitude, it infests both agricultural and natural ecosystems, and known to be noxious to many associated plant species including crops by releasing allelochemicals (Fan *et al*., 2010) [9]. Different parts of *L. camara* (hereafter called lantana) contain allelochemicals, mainly alkaloid and phenolic compounds, which can interfere with seed germination and early seedling growth of many plant species (Sahid and Sugau, 1993; Sharma *et al*., 2005) [2, 5]. The water soluble allelochemicals of lantana were reported to inhibit early seedling growth of *Oryza sativa, Triticum aestivum, Vigna sinensis, V. mungo, V. radiata, Cucurbita pepo, Abelmoschus esculentus, Amaranthus tricolor* and some weeds (Day *et al.*, 2003; Sharma *et al*., 2005; Hossain & Alam, 2010; Nawab and Yogamoorthi, 2016; Julio *et al*., 2019; Anwar *et al*., 2021) [5, 7, 10-13]. Multiple physiological effects including impaired water and nutrient uptake, and decreased shoot turgor pressure caused by some phenolic compounds of lantana were reported by Barkosky and Einhellig (2003) [14].
Lantana is exotic species to Ethiopia that has wide ecological tolerance to successfully grow in various soil types (Day *et al*., 2003) [7]. It exists in abundance in Eastern part of Ethiopia particularly in Dire Dawa, Hararghe and Somali regions in a form of hedge around farm lands and along road sides. In addition, it occupies large expanses of unmanaged land by dominating other native rangeland plant species. With increasing density of lantana in forest and grasslands, declining of species richness is a common phenomenon (Chatanga *et al*., 2008) [15]. Direct negative allelopathic effects of lantana on some crops and its effects on soil quality have been reported previously (e.g., Fan *et al*., 2010; Osunkoya and Perrett, 2010) [9, 16]. However, these effects may vary with plant species and region (Osunkoya and Perrett, 2010). [16] Farmers in Hararghe region of eastern Ethiopia

cultivate different vegetables mainly Brassicaceous species including *Lepidium satvium* L. (Brassicaceae). In Ethiopia, *L. sativum* is known as "fetto" and cultivated for its medicinal and food values (Amare, 1976) [17]. However, information related to the allelopathic effects of lantana on the growth of *L. sativum* is lacking. We hypothesized that lantana leaf extracts and soils invaded by it have negative allelopathic effects on *L. satvium*. Therefore, this research was initiated with the objective of evaluating allelopathic effect of lantana leaf extract and residual toxicity of soils sampled from land invaded by lantana on *L. sativum*. *L. sativum* was selected as a model plant for its short life cycle and sensitivity to allelochemicals besides its great importance as food and medicinal plant in Ethiopia.

Materials and Methods

Plant Material and Soil Sampling Site

In Hararghe region, Eastern part of Ethiopia, *L. camara* is a widely distributed weed that is found mostly around farm lands as hedge and along road sides. It is also seen encroaching to unmanaged areas covered by natural vegetation. Thus, fresh leaves and soil samples were collected from around Haramaya University (9°24'53.13"N and 42°01'55.69"E) East Hararghe zone of Oromia regional state, Ethiopia. Sampling field was a mosaic land covered by thickets of lantana interspersed with patches of small shrubs, grasses and forbs, and subjected to open grazing by livestock. Crops such as sorghum, maize, khat (*Catha edulis*) and vegetables mainly of brassicacious spp. are also cultivated by local farmers adjacently.

Allelopathic plant

In the petri plate testing, *L. camara* served as allelopathic plant and solvent extracts of the leaves were used for testing its inhibitory potential.

Target plant

L. sativum was the target plant and its seeds were collected from the fields of Bate village, Haramaya district.

Design for Lantana Leaf and Soil Sampling

Sampling design used by Fan *et al*. (2010) [9] was followed in this study. Four sampling points that were densely covered by stands of lantana were purposively marked to take leaf and soil samples. The four sampling points (replicates) were far apart from each other by at least 25 m and had roughly similar slope and topography with sites adjacent to them covered by patches of grasses; forbs and other shrubs, but not lantana. From each replicate sampling point, fresh leaves of focal lantana plants were collected and placed in the same plastic bag. Soil samples were also taken from the four sampling points starting from under lantana canopy (UC), and in a distance gradient within 1-2 and 2-3 meter limits outside of the canopy influence in the same direction. Soil samples were taken from the upper 20 cm using an auger in a 1 m x 1 m quadrat from each distance gradient. In each quadrat, soil sample was taken from the four corners and center of the quadrat and pooled to form a composite soil. The sampled soils were used for cultivation of *L. sativum* in pots in greenhouse.

Lantana Leaf Extraction

Lantana leaves sampled from focal plants at for sampling points were first thoroughly washed with distilled water and air dried in the laboratory at room temperature for about ten days. Air dried leaves were powdered together using mortar and pestle. The powder (100 g) was mixed with 300 ml of organic solvents (Ethanol and Hexane separately) and left on the table in the laboratory for 24 hours with intermittent stirring using glass rod (Egigu *et al*., 2010) [18]. After 24 hours of soaking, the extract was filtered under suction using Whatman no.1 filter paper and the solvent in the filtrate was evaporated under reduced pressure using a rotary evaporator at 55°C to remove the solvent and the dried extract was stored in refrigerator until bioassay.

Extracts' Allelopathic Bioassay

In order to test the impact of lantana's leaf extracts on seed germination parameters of *L. sativum*, the dried extracts (5 and 10 g) were reconstituted by dissolving in 100 mL of distilled water to have 5 and 10% (w/v) concentrations. Prior to germination bioassay, seeds of *L. sativum* were surface sterilized using 15% Sodium hypochlorite for 20 min and rinsed in distilled water. Thereafter, 10 seeds were placed on Whatmann No.1. filter paper that received 15 mL of 5 and 10% hexane and ethanolic extracts (separately) in Petri dishes of 9 cm diameter. Distilled water of equal volume to the extract was used as negative control. The seeds were randomly assigned to different treatment levels and Petri dishes were arranged on a table in a laboratory in 5 replicates and incubated for days to complete germination. Petri dishes were made to get more or less the same amount and types of light throughout the incubation period and moved around to avoid position effect. Moisture in the Petri dishes was maintained by adding 2 mL of the extract or distilled water every 2 days. After days of incubation, percent germination and, shoot and root lengths of *L. satvium* were measured.

Impact of Soils Invaded by Lantana

Soil samples of each distance gradient, i.e., UC, 1-2 m and 2-3 m distances outside the influence of lantana canopy obtained from the four sampling points (replicates) were filled in separate plastic pots having surface

area of 380 cm^2. After sowing 10 seeds of the test plant in each pot, the pots were arranged on a table in greenhouse.

The plants were watered with tap water regularly to keep soils always moist, but were not fertilized. The plants were maintained until maturity and parameters such as germination percentage, aboveground plant height measured at harvest, shoot dry weight at harvest, seed yield at harvest and thousand seeds weight were measured eventually.

Statistical Analysis

Data obtained from leaf extract bioassays were analyzed using General linear model to check the impact of extraction solvent and extract concentrations as main effects and their interaction on dependent variables. Effects of soils on growth parameters were analyzed using one-way ANOVA. Statistical software SPSS for Windows 16.0 (SPSS; Chicago, IL, USA) was used to analyze all the data. Differences between means were separated using LSD test and *P*-value less than 0.05 was considered as statistically significant.

Results

Effects of Lantana Leaf Extracts on Germination Percentage of L. sativum Seeds

Extract concentrations and solvents used to make extracts were the main effects. The extracts, at all levels of concentration, significantly (df=2, F= 48.257, P<0.001) inhibited *L. sativum*'s seed germination when compared with control and germination inhibitory effect increased with increasing extract concentration (Fig. 1). Seed germination was also significantly (df=1, F= 26. 737, P<0.001) varied with the type of solvent used to make extraction with hexane extract exhibiting the highest inhibitory effect than ethanolic extract (Fig. 1). That is, hexane extract was superior to ethanolic extract in inhibiting percent seed germination with complete inhibition of seed germination at 10% (w/v) concentration.

Effects on Shoot and Root Lengths of L. sativum

Shoot and root growths of *L. sativum* were significantly (P<0.05) inhibited by extract concentration, extraction solvent and their interaction (Fig. 2 A and B). Shoot and root growth inhibitions were more pronounced with increasing extract concentration. Hexane extracted solution had significantly (P<0.05) higher negative impact than ethanol extracted solution (Fig. 2 A and B).

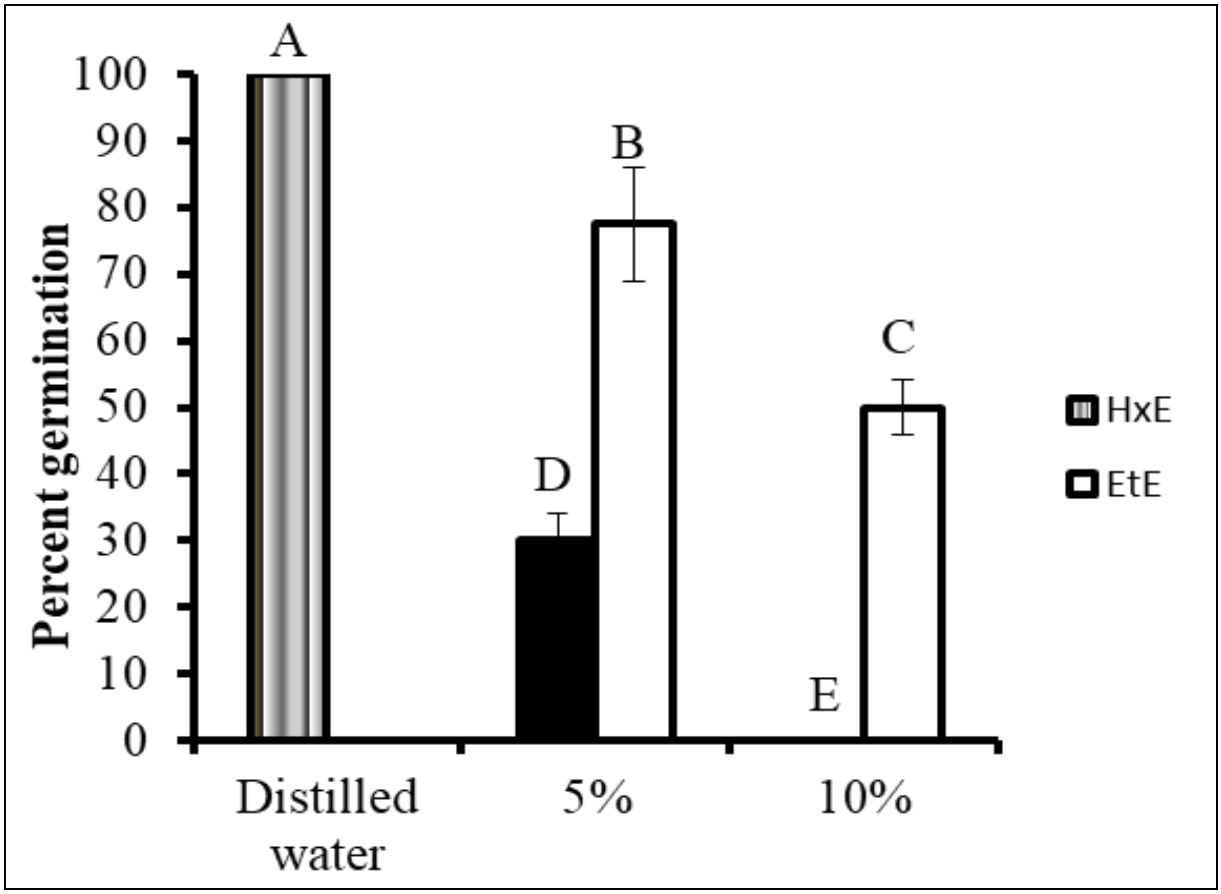

Fig 1: Germination percentage of seeds of *L. sativum* treated with different concentrations of leaf hexane and ethanol extracts of lantana. Values are Mean ± S.E, n=5. *Note*: Black solid bar graph for 10%w/v hexane extract did not appear as value is zero.

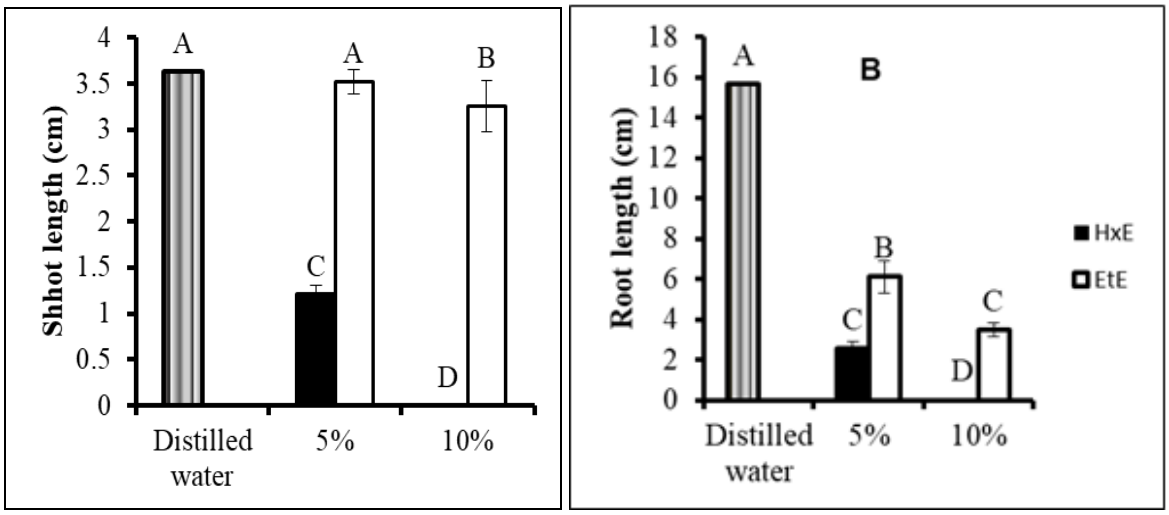

Fig 2: Shoot (A) and root (B) lengths of *L. sativum* treated with different concentrations of leaf hexane and ethanol extracts of lantana. Values are Mean ± S.E, n=5. *Note*: Black solid bar graph for 10%w/v hexane extract did not appear as value is zero.

Impact of Soils from Lantana Invaded Site on Growth of L. sativum

Aboveground plant Height (PH), shoot dry weight (SDW), seed yield (SY) and 1000 seeds weight (TSW) of *L. sativum* grown on soils invaded by lantana were significantly higher than those grown on soils sampled from lantana free sites. However, no significant difference was seen in percent germination (Table 1).

Table 1: Impact of soils sampled from under *L. camara* canopy and lantana free-sites on growth performance and grain yield of *Lepidium sativum.*

	Soil Sampling Position				
Germination parameters	**UC**	**1-2m**	**2-3m**	F-value	P-value
Germination (%)	95.50±1.55[a]	93.00±0.90[a]	93.00±0.90[a]	`0.613	0.62
PH (cm)	47.66±0.96[a]	45.54±1.80[a]	37.61±0.85[a]	27.412	0.07
SDW (gm)	3.91±0.05[a]	2.91±0.10[a]	2.32±0.09[a]	1.538	0.278
SY (kg ha^{-1})	3633.33±120.18[a]	3680.00±75.73[a]	3616.67±2.5[a]	0.146	0.867
TSW	2.07±0.03[a]	2.10±0.06[a]	1.83±0.03[b]	10.83	0.05

Note: Values are Mean ± SE, n=4. UC= Soil sampling position was from under the canopy of lantana; 1-2m= Soil sampling position was within a distance of >1m &<2m outside the influence of lantana canopy; 2-3m= Soil sampling position was within a distance of >2m &<3m outside the influence of lantana canopy; PH=aboveground plant height measured at harvest; SDW=Shoot dry weight at harvest; SY=Seed yield at harvest; TSW=Thousand seed weight. Values followed by similar lower case letters in a row are not significantly different, whereas those represented with different letters are significantly different at $P<0.05$.

Discussion

Leaf Extracts of Lantana are Toxic to L. sativum

Our most significant observations were that direct application of leaf extracts of lantana had negative effects on *L. sativum*'s seed germination and early seedling growth. Inhibitory effects of allelochemical at a germination and early seedling growth stage may predispose crops to other abiotic and biotic stresses, hence reduce their productivity (Dekker and Maggitt, 1983) [19]. Germination and early seedling growth inhibitory effects of direct extract application increased with increasing extract concentration. This suggests that lantana leaf possesses secondary compounds that have negative allelopathic effect directly on the test plant. Direct allelopathic effect of lantana was reported previously by different researchers (e.g., Ahmed *et al*., 2007; Jabeen and Ahmed, 2009; Hossain and Alam, 2010, Desalegn, 2014; Abiyu and Raja, 2015; Rusdy and Ako 2017; Tandoc *et al*., 2019) [20, 21, 10, 22-25] on several other crop species. The phytotoxicity of leaf extract may be attributed to secondary compounds, mainly to complex interaction of the some alkaloids and phenolic compounds (Ambika *et al*., 2003) [26]. In addition to phenolics, report by Kong *et al.* (2006) [27] indicates that triterpenes: lantadene A and B (pentacyclic triterpenoids) isolated from lantana are also potent allelochemicals. The triterpenoids had inhibitory effects on germination, radical growth and the function of photosystem II (Wang *et al*., 2014) [28]. Both hexane and ethanolic leaf extracts significantly reduced percent seed germination and early seedling growth at 5% (w/v) concentration compared to the control. However, hexane leaf extract had more inhibitory effect than ethanolic leaf extract as it completely inhibited percent seed germination at 10% (w/v). This shows that solvents of different polarity have different potential to extract compounds of different profile showing different activities (Egigu *et al*., 2010) [20]. The outcomes of the present study also exhibited that the inhibitory effects of lantana leaf extracts on the germination of *L. sativum*'s seed was concentration dependent and the higher concentration

possessed pronounced inhibitory effects than the lower concentration. The matching tendency was observed in some previous investigations (Eg. Ashrafi *et al*., 2008; Mishra, 2015) [29, 30].

Seed germination may be inhibited due to hampered resource mobilization by allelochemicals during early stages of seed germination (Gniazowska and Bogatek, 2005) [31]. It is also possible that allelochemicals such as some phenolic compounds impair the synthesis and/or activity of gibberellic acid (Einhellig, 1996) [32], which regulates the production of amylase (Chandler *et al*., 1984) [33] so that seed germination is negatively affected. Einhellig (1996) [32] reported that allelochemicals decrease elongation, expansion and division of cells, which are growth prerequisites. The inhibition of shoot and root lengths by lantana extracts also suggests the occurrence of some allelochemicals that interfere with important metabolic activities capable of promoting cell division and elongation. Sasikumar *et al*. (2002) [34] reported the inhibitory effects of phenolic compounds on phosphorylation pathway, Mg activation and ATPase activity. They also mentioned that allelochemicals might decrease synthesis of total carbohydrates, proteins and nucleic acids (DNA and RNA) hence negatively affect growth. Compared to the control, root length was six-fold reduced, whereas shoot growth was reduced by three-fold only at 5% w/v of leaf hexane extract. This may be attributed to more impairment of endogenous plant growth hormones synthesis and/or activity in the root by allelochemicals.

Impact of Soils from Lantana Invaded Site on Growth of L. sativum

Allelochemicals may enter into soils invaded by the donor plant through different means including tissue decomposition, aerial leachates from a donor plant and exudation from roots. We expected that these chemicals will also have inhibitory effect after entering into the soil. However, lantana invaded soils had no significantly different effects on growth parameters measured as compared to soils used from lantana free sites. The fact that soils invaded by lantana had no inhibitory effect on test plant suggests less accumulation of allelochemicals in the soils. It is also possible that allelochemicals might have been degraded or transformed into some other non-toxic forms (Inderjit and Weiner 2001; Cseke *et al*., 2006; Albuquerque *et al*., 2010) [35-37] in soils by microbial and physicochemical processes. Achhireddy and Singh (1984) [38] and Sahid and Sugau (1993) [2] reported that soils invaded by lantana have no inhibitory effect when used to grow other crops as opposed to direct extract application to the seeds. Apart from direct toxicity to recipient plant, allelochemicals may have negative influence on the amount and availability of plant essential nutrients in the soil, hence reduce plant's growth. However, previous studies by Fan *et al*. (2010) [9] and Osunkoya and Perrett (2010) [16] showed that invasion of soil by lantana has no negative effect at least on some major soil macro-nutrients such as phosphorus, nitrogen and organic carbon. Therefore, although not tested in this study, it is less likely that soil invasion by lantana has negative impact on soil nutrient status to reduce growth performance of the tested plant. In conclusion, this study showed that lantana leaf extracts hamper germination and early seedling growth of *L. sativum*, but the soils invaded by lantana has no negative impact on *L. sativum* growth and seed yield.

Conclusions

A petri dish study was conducted to assess the retarding effects of extracts of *L. camera* leaf and soil invaded by it on the germination and early seed growth of *L. sativum*. The outcomes of the study revealed that the target species *L. sativum* was much sensitive to hexane extract of lantana than to the ethanolic extract. The activity of the both extracts was found as concentration dependent. The soil invaded by lantana exhibited no retarding effects on the seed germination of *L. sativum*. The experiment reveals that lantana leaves are potential source of allelochemicals. Further studies, under field conditions and using latest techniques, are required to examine the effect of lantana on specific crops and weeds.

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