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EFFECT OF WEDELIA TRILOBATA ON THE GERMINATION AND SEEDLING GROWTH OF COWPEA

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Abstract: *Wedelia trilobata* is a noxious weed. In this study we found out the impact of different leachates(both fresh plant leachate and leachate from dried plant) on the germination and seedling growth of cowpea. Different concentrations of leachate(2%,4%,6%,8% and 10%) were used, distilled water used as control. Seeds were sown in these leachate in petriplates and germination parameters were measured. All the germination parameters (except germination percentage and response index) have no significant variation. Whereas radicle length was high in dry plant leachate compared to fresh leachate. Also phytochemical screening revealed both leachate contain most of the secondary metabolites such as alkaloids, flavonoids, saponins, tannins, triterpenoids, anthraquinone glycosides, coumarins, phenols, volatile oils, quinones and anthraquinones.

IndexTerms:cowpea,leachate,secondary metabolite,*Wedelia trilobata*

I. INTRODUCTION

Allelopathy is defined as the direct harmful or beneficial effect of one plant on another through the production of chemical compounds that escape into the environment (Brown et al., 1991). Allelochemicals are defined as bio communicators, suggesting the possibility of active mixtures, because of the increasing number of findings in which single compounds are not active or not as active as a mixture. Allelopathy arises from the release of chemicals by one plant species, which may affect other species in its vicinity, usually to their determinant. These chemicals can be classified as secondary plant metabolites (such as terpenoids, phenolics, alkaloids, isoprenoids, flavonoids and gluconolates etc.). Allelochemicals can be seen in virtually all plant tissues, including roots, stem, fruits, flowers, leaves, pollen and seeds. *Wedelia trilobata* is an invasive weed, makes monospecific strand and suggests the need of remarkably potent mechanisms. *Wedelia trilobata* is widely spread in many tropical and subtropical areas and considered as a serious weed due to its rapid growth rate. It is very difficult and expensive to control growth of this plant in the widespread agricultural areas. *Wedelia trilobata* is an attractive source of many secondary metabolites. Its phytotoxic effects on crops and several weeds have previously been described (Zhang et al., 2004; Nie et al., 2004; Dai et al., 2016). Reports revealed that rice (*Oriza sativa*) would have poor growth and low yield after using of *W. trilobata* as green manure (Nie et al., 2004). However, prior allelopathic study revealed that *W.trilobata* allelochemicals are not toxic to leguminous crop species and also observed that germination bioassays are suitable for the allelopathic analysis of *W.trilobata*. Therefore the current study intents to do the preliminary phytotoxic analysis of leachate extracted from *W. trilobata* on cowpea (*Vigna unguiculata*) by using laboratory bioassays.

II. MATERIALS AND METHODS

Preparation of plant leachate

Leachate prepared from fresh plants and dried plants (air dried and powdered). For the preparation of leachates, freshly chopped plants (represented as fresh leachate) and plant powder (represented as dry leachate) soaked in distilled water at 1:10 (w/v) under aseptic conditions for one day (24h) two days (48h) and three days (72h) separately at room temperature. After that the leachates were filtered through Whatman No.1 filter paper to remove debris and checked the pH of the leachate. Filtrate stored in room temperature until further analysis. Samples were denotes as fresh leachate and dry leachate.

Tests were conducted for preliminary phytochemical analysis to find out various allelochemicals present in the leachate (fresh plant leachate and dry plant leachate) samples.

Preliminary phytochemical analysis

The following methods were adopted to detect major metabolites in the leachate samples.

Detection of alkaloids (Harborne, 1984):

a. **Dragendorff's test**: Dragendorff's reagent was added to 2ml of the test solution. Orange precipitate indicated the presence of alkaloids.
b. **Mayer's test**: One or two drops of Mayer's reagent were added to 2ml of the test solution. White precipitate indicated the presence of alkaloids.
c. **Wagner's test**: Two drops of Wagner's reagent was added to 2ml of the test solution. Alkaloids gave brown precipitate.

Detection of flavonoids (Harborne , 1984):

a. **Alkaline reagent test**: Added five drops of 5% NaOH to 2ml of the test solution. Increase in intensity of yellow colour, which become colourless on addition of a few drops of 2M HCl, indicated the presence of flavonoids.
b. **Lead acetate test**: Added few drops of 10% lead acetate to 2ml of the test solution. Formation of yellow precipitate indicated the presence of flavonoids.

Detection of saponins (Trease et al., 1989):

Foam test: Five ml of the test solution was taken in a test tube and shaken well for 5minutes. Formation of stable foam indicated the presence of saponins.

Detection of tannins (Trease et al., 1989):

a. **Ferric chloride test**: Added few drops of 5% ferric chloride solution to 2ml of the test solution. Tannins gave dark green or blue colour.
b. **Gelatin test**: Added 5 drops of 1% gelatin and 10% NaCl to 2ml of the test solution. Formation of white precipitate indicated the presence of tannins.

Detection of steroids

Salkowski test: Two ml of the test solution was shaken with 1ml chloroform and added few drops of concentrated H_2SO_4 along the side of the test tube. A red-brown colour at the interface indicated the presence of triterpenoids.

Detection of cardiac glycosides

Keller-Killiani test: Added 0.4 ml of glacial acetic acid and a few drops of 5% $FeCl_3$ solution to 2ml of the test solution. Further 0.5ml of concentrated H_2SO_4 was added along the side of the test tube. The formation of blue colour in acetic acid layer confirmed the test.

Detection of anthraquinone glycosides

Hydroxyanthraquinone test: To 1ml of test solution, added few drops of 10% KOH. Formation of red colour confirmed the test.

Detection of proteins

Biuret test: To 2ml of the test solution 5 drops of 1% $CuSO_4$ solution and 2ml of 10% NaOH were added and mixed thoroughly. Formation of purple or violet colour indicated the presence of proteins.

Detection of phenol (Harborne, 1998)

$FeCl_3$ test: Dissolved 2ml of the test solution in 5ml distilled water and added few drops of neutral 5% $FeCl_3$ solution. A dark green colour indicated the presence of phenol.

Detection of volatile oils

Two ml of the test solution was shaken with 0.1ml of NaOH and a small quantity of HCl. Volatile oils gave a white precipitate.

Detection of quinones

Few drops of NaOH was added to 2ml of the test solution. A blue/green/red colour indicated the presence of quinones.

Detection of anthraquinones

Added 2 ml of 25% NH_3 solution to 2ml of the test solution. A cherry red colour indicated the presence of anthraquinones.

Detection of carbohydrates

Molisch's test: dissolved 2 ml of the test solution in 10ml distilled water and added 2 drops of 2% of ethanolic extract of alpha naphthol and 2ml concentrated H_2SO_4. Reddish violet at the junction indicated the presence of carbohydrate.

Detection of coumarins (Harborne, 1984):

Added 3ml 10% NaOH to 2ml of the test solution. Yellow colour indicated the presence of coumarins.

Detection of fat and fixed oils

To 1ml of the test solution, added 1% $CuSO_4$ and a few drops of 10% NaOH. Formation of clean blue colour confirmed the test.

Germination of seeds in leachate

Seeds were surface sterilized with Sodium hypochlorite solution (0.1%) for 5 minutes. Then washed in distilled water for several times and soaked in distilled water for 2 hours. Filter paper was used for germination tests. Five concentrations (2, 4, 6, 8 and 10 %) of leachate solutions prepared by adding water to the concentrated stock leachate. Control was kept without leachate. Germination was evaluated by placing 15 seeds in a 9 cm diameter and 1. 5 cm depth petri dish containing one layer of sterilised filter paper, moistened with 10 ml of each test leachate solutions and paper with distilled water served as control. Petri dishes sealed with parafilm and maintained at (26±3°C) room temperature for ten days. Data were recorded at an interval of 24 h. in laboratory. Experiment was repeated eight times and recorded the data statistically.

Germination percentage (GP), germination rate (GR), coefficient rate of germination (CRG), uniformity of germination time (UGT), response index (RI), and seedling vigour index (SVI) were calculated. The root and hypocotyl lengths were measured with a ruler. Data were subjected to statistical analysis ANOVA.

III. RESULTS AND DISCUSSION

Wedelia trilobata has been recognized as a serious invasive weed. Once it is established in plantations, overgrows and develops into a thick ground cover, thus crowding out or preventing the regeneration of other species. An earlier study indicated that *W. trilobata* has strong vegetative propagation and its stem exhibits high plasticity (Wu et al., 2005).

Estimation of allelochemicals from leachate

Soaking time was standardised as two days (48 hours) for leachate preparation. The various phytochemicals detected in the fresh and dry leachate did not show any variation and are represented in the Table: 1as fresh and dried plant leachate. The pH of leachate ranging between 5-6, slightly acidic in nature. In the present study preliminary phytochemical analysis to find out the phytochemicals present in the leachate revealed the presence of major secondary metabolites such as alkaloids, flavonoids, saponins, tannins, triterpenoids, anthraquinone glycosides, coumarins, phenols, volatile oils, quinones and anthraquinones, whereas cardiac glycosides were absent. Carbohydrates, proteins, fats and fixed oils were totally absent in the leachate. Fresh plant and dried plant leachates possess same phytochemicals. Studies reported that the main secondary metabolites of *Wedelia trilobata* mainly consist of terpenoids, flavonoids, polyacetylenes, steroids, sesquiterpenoids, triterpenoids, diterpenoid and benzene derivatives (Bohlmann et al., 1981; That et al., 2007; Wu et al., 2010; Qiang et al., 2011). It has been reported that the aerial parts and leaves of *W. trilobata* contain diterpenes, sesquiterpenes and triterpenes (Balekar et al., 2012; Ren et al., 2015). The flower parts contain monoterpenes (Shankar and Thomas, 2014; Husain and Kumar, 2015) and the root contains sesquiterpenes such as caryophyllene and germarcrene D (Verma and Khosa, 2015). In particular, the high diterpene levels in *W. trilobata* are associated to the invasive ability of the plant to adapt to different habitats (Dai et al., 2016). Other studies reported that the leaves and stem contains eudesmanolide lactones, luteolin and kaurenoic acid (Block et al., 1998; Govindappa et al., 2011).

Table No. 1: Phytochemical evaluation in different leachates

Metabolites	Presence of metabolite in	
	fresh plant leachate	dried plant leachate
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Triterpenoids	+	+
Cardiac glycosides	-	-
Anthraquinone glycosides	+	+
Coumarins	+	+
Phenols	+	+
Volatile oils	+	+
Quinones	+	+
Anthraquinones	+	+
Carbohydrates	-	-
Proteins	-	-
Fat and fixed oils	-	-

+ : present; - : absent.

Germination of seeds in leachate

After standardising the protocol for individual assay, germination experiment was conducted with cowpea and the result revealed that there is no inhibitory effect of leachate on germination.Germination was 100% in all samples except TD_4 and TD_5. However these samples also showed above 85% germination. Germination rate (GR) is the speed of germination. High germination rate was noted in all the leachate treatments. High germination rate means more seeds germinated in shorter time. Within 3 to 5 days all the seeds were germinated in all the treatments as well as control. Coefficient rate of germination (CRG) was not significantly affected by treatment. Uniformity of Germination Time (UGT) was rather same in fresh leachate experiment whereas it was slightly increased in dry leachate dose TD_3 compared to others and control. In case of response index (RI) control as well as treatments showed the value 0, this indicates no response on treatment, however two doses TD_4 and TD_5 showed values below 1. Seedling Vigour Index (SVI) was very high in TD_4 compared to all other samples. Effect of *Wedelia trilobata* leachate on germination of cow pea is documented in Table 2. From the present study it is suggested that the applied doses do not have any inhibitory effect on germination of cowpea. It is also evidenced from the further germination parameters such as germination rate (GR), Coefficient rate of germination (CRG), uinfirmity of germination time (UGT) and response index (RI). Result also revealed that seedlings are very healthy when compared to control. However radicle growth was more in dry plant leachate samples compared to fresh leachate samples. Very long root (14.07 cm) was observed in all the seeds germinated in TD_2 followed by TD_3 and TD_4(table 3).

The previous studies reported on the germination inhibition by the leaf and leaf litter of *W.trilobata* on lettuce (Wu et al., 2008) and rape (Zhang et al., 2013). Azizan et al. (2019) reported that .increasing the concentration of *W. trilobata* extracts significantly inhibited *Lactuca sativa* germination rate, shoot height and root length. These findings revealed growth inhibitory effect of the extract and also indicate the presence of phytotoxic substances. Dai et al., (2016) reported that the *W. trilobata* leaf extracts had allelopathic effects on seed germination and seedling growth of invasive and non-invasive competitors. These reports suggest that the allelopathic effect may be target species specific and cowpea is highly tolerant to this effect.

The allelopathic potential of aqueous extracts of *Wedelia trilobata* on rice (*Oryza sativa* L.) was studied (Nei et al., 2005) and reported that the aqueous extracts of root, stem, leaf and of the whole plant of *W. trilobata* reduced the activities of some hydrolytic enzymes and some protective enzymes in geminating rice seeds. Nei et al., (2005) also reported that geminating rice seeds treated with *W. trilobata* extract had higher membrane permeability, lower respiratory rate and vitality. Dry plant leachate may contain high concentration of allelochemicals compare to fresh plant leachate. However most of the volatile constituents may be lost from dry samples. Phytochemical evaluation of fresh and dry plant leachate revealed the presence of similar major groups of phytochemicals . From all the germination parameters it is clear that the applied doses (2-10 %) of leachate treatment not adversely affect germination and seedling growth of cow pea.

Table No. 2: Effect of leachate on germination of cow pea

Leachate	Code	GR	GP	CRG	UGT	RI	SVI
Fresh plant leachate	Control	5.00	100	1.79	0.40	0	852
	TF_1	4.67	100	2.29	0.40	0	869
	TF_2	5.00	100	1.73	0.40	0	603
	TF_3	3.67	100	1.68	0.40	0	684
	TF_4	4.17	100	1.60	0.42	0	648
	TF_5	3.61	100	1.79	0.41	0	854
Dry plant leachate	Control	6.05	100	0.67	0.49	0	1125
	TD_1	6.10	100	0.63	0.49	0	1262.76
	TD_2	4.67	100	0.67	0.49	0	2402
	TD_3	3.67	100	0.79	0.56	0	1152
	TD_4	3.25	93.33	1.38	0.49	0.85	1291.69
	TD_5	2.98	86.67	1.83	0.49	0.52	866.70

Results are presented as mean (n=10). TF_1: fresh leachate 2%; TF_2: fresh leachate 4%; TF_3: fresh leachate 6%; TF_4: fresh leachate 8%; TF_5: fresh leachate 10%; TD_1: dry leachate 2%; TD_2: dry leachate 4%; TD_3: dry leachate 6%; TD_4: dry leachate 8%; TD_5: dry leachate 10%; GR: Germinarion Rate; GP: Germination Percentage; CRG: Coefficient Rate of Germination; UGT: Uniformity of Germination Time; RI: Response Index; SVI: Seedling Vigour Index.

Table No. 3: Effect of leachate on seedling growth and seedling vigour index of cow pea

Type of leachate	Treatment	Root length	Embryo axis length(cm)	Shoot length	Seedling length
Fresh plant leachate	Control	3.34 ± 0.19^{ab}	4.71 ± 0.38^{a}	1.32 ± 0.18^{a}	9.37 ± 0.51^{a}
	TF1	4.69 ± 1.04^{a}	5.52 ± 0.56^{a}	1.27 ± 0.23^{a}	11.48 ± 1.57^{a}
	TF2	2.68 ± 0.58^{b}	4.04 ± 0.66^{a}	1.28 ± 0.16^{a}	8.00 ± 1.27^{a}
	TF3	2.36 ± 0.44^{a}	4.41 ± 0.64^{a}	0.99 ± 0.31^{a}	7.76 ± 1.25^{a}
	TF4	2.56 ± 0.45^{a}	4.74 ± 0.64^{a}	1.00 ± 0.21^{a}	8.30 ± 1.22^{a}
	TF5	4.65 ± 0.54^{a}	5.51 ± 0.42^{a}	1.19 ± 0.21^{a}	11.35 ± 0.87^{a}
Dry plant leachate	Control	4.01 ± 0.57^{c}	4.80 ± 0.33^{bc}	2.44 ± 0.21^{a}	11.25 ± 0.75^{b}
	TD1	5.78 ± 0.98^{bc}	5.70 ± 0.45^{b}	2.05 ± 0.33^{ab}	13.53 ± 1.17^{b}
	TD2	14.07 ± 1.65^{a}	7.08 ± 0.44^{a}	2.87 ± 0.35^{a}	24.02 ± 1.94^{a}
	TD3	8.34 ± 1.56^{b}	4.73 ± 0.34^{bc}	1.33 ± 0.43^{bc}	14.40 ± 1.61^{b}
	TD4	8.34 ± 1.35^{b}	4.15 ± 0.47^{cd}	1.35 ± 0.33^{bc}	13.84 ± 1.97^{b}
	TD5	6.31 ± 0.84^{bc}	3.16 ± 0.42^{d}	0.53 ± 0.20^{c}	13.84 ± 1.97^{b}

Results are presented as mean ± SEM (n=10). Means denoted with different superscript letters differ significantly at P>= 0.05 based on Duncan's multiple range test. TF_1: fresh leachate 2%; TF_2: fresh leachate 4%; TF_3: fresh leachate 6%; TF_4: fresh leachate 8%; TF_5: fresh leachate 10%; TD_1: dry leachate 2%; TD_2: dry leachate 4%; TD_3: dry leachate 6%; TD_4: dry leachate 8%; TD_5: dry leachate 10%.

Figure 1: Fresh plant leachate. Seedlings **1**: control; Seedlings **2**: leachate-2%; Seedlings **3**: leachate 4%; Seedlings **4**: leachate-6%; Seedlings **5**: leachate-8%; Seedling **6**: leachate-10%.

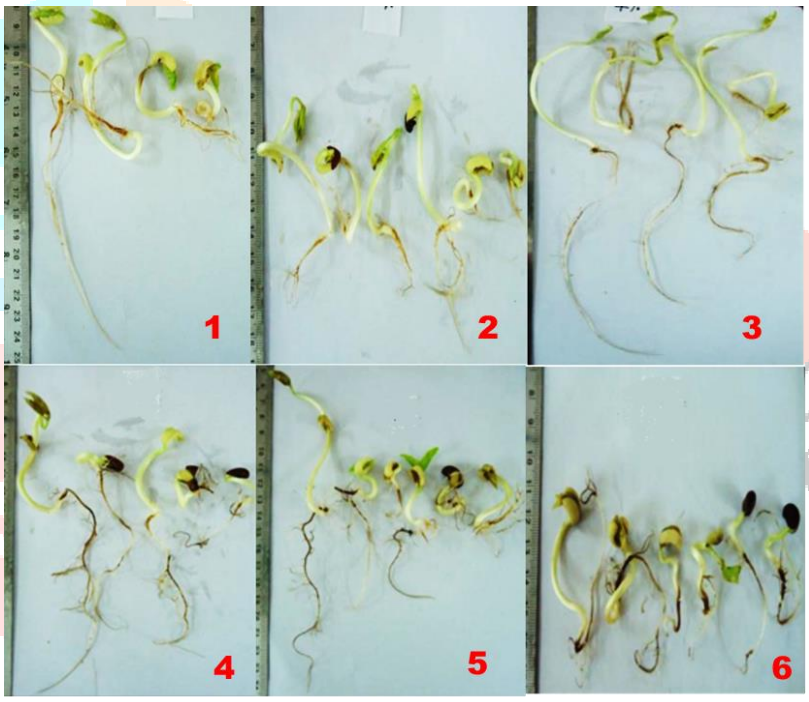

Figure 2: Dry plant leachate. Seedlings **1**: control; Seedlings **2**: leachate 2%; Seedlings **3**: leachate- 4%; Seedlings **4**: leachate-6%; Seedlings **5**: leachate-8%; Seedlings **6**: leachate- 10%.

IV CONCLUSION

Preliminary phytochemical screening revealed that *Wedelia trilobata* plant contain most of the secondary metabolites,primary metabolites are absent. Leachate have no negative impact on cowpea germination and its effect is not dose dependent. But some of the germination parameters in treatment shown a slight variation compared control. Seedling growth and seedling vigour index of cowpea showed that leachate at particular dose enhances the seedling growth. Certain toxic effect was exist in leachate at higher doses however not at high significant level.

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