Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/12807
DOI URL: http://dx.doi.org/10.21474/IJAR01/12807

ISSN NO. 2320-5407

RESEARCH ARTICLE

EFFECT OF *WEDELIA TRILOBATA* ESSENTIAL OIL ON THE GERMINATION AND SEEDLING GROWTH OF COWPEA

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Manuscript Info

Manuscript History
Received: 05 March 2021
Final Accepted: 09 April 2021
Published: May 2021

Key words:-
Cowpea, Essential Oil, GC-MS Analysis, *Wedelia trilobata*

Abstract

Wedelia trilobata is an invasive weed of the family Asteraceae.Objective of the study was to find out the impact of essential oil on the germination and seedling growth of cowpea. Different concentrations of essential oil(200, 400, 600, 800 and 1000ppm) were used,a controlwas kept without oil. Cowpea seeds were sown in these essential oil in petriplates,at the end of test period all the germination parameters were measured. All the germination parameters showed that treatment had no negative impact on germination. While root length,shoot length,embryo axis length,seedling length showed a significant variation in treatments compared to control. GC-MS analysis of essential oil revealed that it is a mixed chemotype and α-pinene (10.859 %), caryophyllene oxide (5.325%) and spathulenol (7.911%) are the major compounds.

Introduction:-

Wedelia trilobata (L). Hitch. (Asteraceae) (syn. *Sphagneticola trilobata*), is a common weed, widely distributed in India. It is considered as a noxious weed because of fast spreading ability, allelopathic effect on other plants and strong competitiveness with crops. *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). Various studies reported that this plant had impact on germination and growth of different plant species.Therefore the current study intents to do the preliminary phytotoxic analysis of essential oil extracted from *W. trilobata* on cowpea (*Vigna unguiculata*) by using laboratory bioassays.

Materials And Methods:-

Procurement of plant material

The donor plant *Wedelia trilobata* were collected from different places from Thiruvananthapuram and the receiver plant seeds(cowpea)were purchased from Agricultural College, Vellayani, Thiruvananthapuram.

Extraction of essential oil

Essential oil was extracted from 100g of freshly chopped plant material by hydro distillation in a Clevenger apparatus. The oil obtained through hydro distillation was collected in an airtight container (glass tube), dried over anhydrous Sodium sulphate, and stored refrigerator (4° C) until use.

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Characterization of essential oil components:
GC-MS analysis was done using the volatile oil obtained through hydro distillation. GC-MS analysis was carried out at Inter University Instrumentation Centre (IUIC), Mahatma Gandhi University, Kottayam. Volatile oil was injected into Gas chromatography –Mass spectrophotometer. The Gas Chromatography was carried out in GC Clarus 500 Perkin Elmer with column Elite – 5ms (5% Phenyl 95% Dimethyl polysiloxine). Mass detector used was Turbo mass – gold – Perkin Elmer and Nitrogen was used as a carrier gas. Temperature of column was maintained at (220-280ºC).

Germination of cowpea in essential oil
The extracted oil diluted to different concentrations (200, 400, 600, 800 and 1000ppm) and used for germination study. By dissolving oil in water, first dissolveessential oil in sufficient quantity of Tween 20 (1:1) and diluted to 200, 400, 600, 800, 1000 ppm with water. Five petriplates were taken with Whatman No: 1 filter paper as the base and different concentrations of oil were added to each petriplates. Five seeds were sown in each petriplates, then all the petriplates were sealed with parafilm and kept for 7 days under laboratory conditions. Control was kept without oil. Observations were made for one week. Codes were given to oil treated cow pea. ED -200, ED -400, ED-600, ED-800 and ED-1000 respectively to different concentration of oil treated cowpea and control.

All the germination experiment were 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 by using centimetre scale.

Data were analysed by one-way analysis of variance (ANOVA). All the values are expressed as mean value ± SEM. Paired comparison between the groups were made by Duncan's Multiple Range Test. 'p' values of 0.05 or less were considered significant.

Results:-

Estimation of volatile allelochemicals from essential oil
Essential oil was orange-yellow in colour and the odour of the oil herbaceous with medicinal topnote. Quantity obtained from sample showed an average of 3.3 ml\Kg. GC-MS analysis revealed that essential oil is mixed chemotype. Total of 85 compounds were identified and revealed that α-pinene (10.859 %), caryophyllene oxide (5.325%) and spathulenol (7.911%) are the major compounds.This is listed in Table No.1.

Table No. 1:- Identified components of essential oil.

No.	Name of compound	% ge	RT
1	Ethanone, 1-(2-methyl-2-cyclopenten-1-yl)-	0.047	8.103
2	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	0.026	9.533
3	Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv.	0.032	9.744
4	α-Pinene	10.859	9.989
5	Camphene	0.381	10.586
6	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	0.092	10.759
7	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	0.169	11.501
8	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	0.416	11.635
9	β-Myrcene	0.057	12.194
10	Cyclohexane, 1-methyl-3-(1-methylethylidene)-	0.104	12.731
11	Octanal	0.67	13.010
12	Benzene, 1-methyl-2-(1-methylethyl)-	2.437	13.397
13	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate	3.311	13.551
14	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1α,2β,5α)]-	0.279	16.282
15	trans-p-Mentha-2,8-dienol	0.132	16.718
16	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-	0.649	16.859
17	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	0.189	17.192
18	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1α,3α,5α)]-	0.507	17.296
19	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1α,2β,5α)]-	2.554	17.474
20	2(10)-Pinen-3-one, (±)-	0.249	17.967

21	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	0.503	18.558
22	Benzenemethanol, α,α,4-trimethyl-	0.456	18.789
23	3-Cyclohexene-1-methanol, α,α4-trimethyl-	0.811	19.033
24	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	0.165	19.166
25	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	1.537	19.358
26	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	0.778	19.744
27	3-Methyl-4,7-dioxo-oct-2-enal	0.557	19.847
28	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-	0.285	20.128
29	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	0.455	20.448
30	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	2.043	20.587
31	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	0.286	21.064
32	(1R,2R,3S,5R)-(-)-2,3-Pinanediol	0.624	21.291
33	Camphenol, 6-	0.568	21.799
34	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	1.372	22.010
35	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1α,3α,5α)]-	0.549	22.275
36	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-	5.821	22.421
37	(1S,2S,3R,5S)-(+)-Pinanediol	4.446	22.696
38	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	1.656	22.882
39	Cyclohexanone, 2,3-dimethyl-2-(3-oxobutyl)-	0.804	23.018
40	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	0.294	23.271
41	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	0.796	23.372
42	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	0.217	23.528
43	Hydroxy-α-terpenyl acetate	0.391	23.719
44	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-	0.124	23.850
45	2,3-Bornanediol	0.253	23.940
46	Cycloundecanone	0.117	24.036
47	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)-	0.186	24.124
48	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, trans-	0.203	24.170
49	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	0.131	24.248
50	Octanoic acid, 2-propenyl ester	0.909	24.319
51	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	0.946	24.506
52	2,3-Bornanediol	2.319	24.683
53	Cycloundecanone	0.137	24.859
54	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)-	1.006	25.098
56	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, trans-	2.266	25.315
57	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-	0.416	27.072
58	Octanoic acid, 2-propenyl ester	0.878	27.187
59	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	0.460	27.734
60	Caryophyllene oxide	0.294	28.603
61	Longipinocarveol, trans-	0.505	28.710
62	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	0.372	28.835
63	(-)-Spathulenol	**7.911**	29.238
64	Caryophyllene oxide	5.325	29.354
65	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	3.139	30.009
66	1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7a-tetramethyl-	4.159	30.300
67	α-Cadinol	1.240	31.046
68	cis-Z-α-Bisabolene epoxide	0.803	32.539
69	Isoaromadendrene epoxide	1.411	32.773
70	Diepicedrene-1-oxide	1.314	33.804
71	Platambin	2.931	35.545
72	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl	0.395	36.258

73	Murolan-3,9(11)-diene-10-peroxy	0.772	36.379
74	Aristolene epoxide	0.606	36.548
75	Murolan-3,9(11)-diene-10-peroxy	0.678	37.607
76	Androst-2,16-diene	0.872	38.081
77	Phytol	0.766	40.285
78	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-	0.576	40.834
79	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl-, methyl	0.431	41.714
80	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9-(acetyloxy)-3-[(acetyloxy)methyl]…	0.303	41.936
81	16βH-Kauran-16-ol	0.549	42.748
82	δ-Selinene	0.249	42.881
83	dl-3Beta-hydroxy-d-homo-18-nor-5alpha,8alpha,14beta-androst-13(17a)-en-17-one	0.163	44.075
84	1-Naphthalenepentanol, decahydro-5-(hydroxymethyl)-5,8a-dimethyl-γ,2-bis(methylene)-, (1α,4aβ,5α,8aα)-	0.199	44.698
85	Retinoic acid	0.576	45.893

Effect of essential oil on germination of cowpea

Germination parameters and seedling growth suggest that essential oil in this selected dose range did not cause any negative impact on germination of cowpea. Germination rate and germination percentage was not affected by the treatment. Within 3 to 5 days all the seeds were germinated in all the treatments and control.Coefficient rate of germination was found to be same for all the treated and the control seeds. UGT and RI also showed the same values (Table No.2). Length of embryo axis,shoot and root of cowpea showed significant variation among the treatments compared to control. Also seedling length was high in treatments compared to control (Table No. 3). Seedlings raised from essential oil treatment in Figure No.1.

Table No.2:- Effect of essential oil on germination of cow pea.

Experiment	Code	GP	GR	CRG	UGT	RI
Essential oil in different doses (ppm)	Control	100	4.67	0.24	0.7	-
	ED-200	100	3.83	0.24	0.7	0
	ED-400	100	3.17	0.24	0.7	0
	ED-600	100	4.33	0.21	0.7	0
	ED-800	100	4.00	0.21	0.7	0
	ED-1000	100	4.50	0.24	0.7	0

Values are mean of six independent experiments. GP: Germination Percentage; GR: Germinarion Rate; CRG: Coefficient Rate of Germination; UGT: Uniformity of Germination Time; RI: Response Index.

Table No. 3:- Effect of essential oil on seedling growth.

Expt	Essential oil in ppm	Root length(cm)	Embryo axis length(cm)	Shoot length(cm)	Seedling length(cm)
Essential oil in different doses	Control	1.21 ± 0.42^{bc}	0.2 ± 0.02^{a}	0.5 ± 0.00^{a}	2.01 ± 1.09^{e}
	ED-200	3.29 ± 1.09^{ab}	3.48 ± 0.96^{a}	1.14 ± 0.37^{a}	13.18 ± 3.0^{b}
	ED-400	1.09 ± 0.41^{bc}	2.38 ± 0.87^{a}	0.87 ± 0.30^{a}	8.68 ± 2.6^{c}
	ED-600	5.61 ± 1.77^{a}	2.61 ± 1.03^{a}	1.18 ± 0.33^{a}	15.66 ± 5.7^{a}
	ED-800	0.90 ± 0.27^{bc}	0.3 ± 0.01^{a}	1.02 ± 0.28^{a}	3.20 ± 0.35^{d}
	ED-1000	1.94 ± 0.46^{bc}	2.55 ± 0.81^{a}	1.07 ± 0.36^{a}	7.94 ± 2.52^{c}

Values are mean of six replicates ± SD. Means denoted with different superscript letters differ significantly at P>= 0.05 based on Duncan's multiple range test.

Figure No. 1:- Seedlings of cowpea raised from essential oil treatment.

Seedlings raised from different doses of essential oil- Seedling 1: 200ppm; Seedling 2: 400ppm; Seedling 3: 600ppm; Seedling 4: 800ppm; Seedling 5:1000ppm; Seedling 6: control

Discussion:-

Wedelia trilobata aerial parts contain monoterpenes, diterpenes, sesquiterpenes and triterpenes (Balekar et al., 2012; Ren et al., 2015; Shankar and Thomas, 2014; Husain and Kumar, 2015) and the root contains sesquiterpenes (Verma and Khosa, 2015). da Silva et al. (2012) identified fourteen volatile components from the essential oils of *W.trilobata* and characterized as high percentage of sesquiterpenes (25.5-86.4%), monoterpenes (22.9-72.3%) and low levels of oxygenated sesquiterpenes (0.0- 7.4%). They reported germacrene D (11.9-35.8%), α-phellandrene (1.4-28.5%), α-pinene (7.3-23.8%), E-caryophyllene (4.6-19.0%), bicyclogermacrene (6.0-17.0%), limonene (1.8-15.1%) and α-humulene (4.0-11.6%) are the major components.

The experiment to evaluate the effect of essential oil on germination of cowpea under laboratory condition revealed that essential oil not influenced the germination of cowpea. Higher doses also showed 100% germination revealed that essential oil components not inhibited the germination and seedling growth of cow pea.Many studies (Abrahim et al., 2000; Kordali et al., 2007) reported that 1, 8-cineole and camphor have strong phytotoxic effects against various plant species; citronellal, citronellol, linalool (Singh et al., 2002; Singh et al; 2006), α-pinene (Abrahim et al., 2000; Singh et al; 2006) and limonene (Abrahim et al., 2000) are known as high inhibitors of seed germination and seedling growth. In several papers point out that monoterpene hydrocarbons possess lower inhibitory activity than oxygenated compounds (Vokou et al., 2003; Kordali et al., 2007; Singh et al., 2002). Monoterpenes are thought to be important allelopathic agents in hot, dry climates where they act in the vapor phase, because the high vapor density of the essential oils may penetrate into the soil, affecting adversely the growing plants (Scrivanti et al., 2003). Present study revealed that essential oil of *Wedelia trilobata* is safe for cowpea germination but further field study is crucial for its action in the soil medium.

Acknowledgement:-

The authors are thankful to Head, Department of Botany, University of Kerala, Kariavattom for providing facility to do the work.

References:-

1. Abrahim, D., Braguini, W. L., Kelmer-Bracht, A. M.,& Ishii-Iwamoto, E. L. (2000):Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. *Journal of chemical ecology*, *26*(3), 611-624.
2. Balekar, N., Katkam, N. G., Nakpheng, T., Jehtae, K., & Srichana, T. (2012). Evaluation of the wound healing potential of *Wedelia trilobata* (L.) leaves. *Journal of Ethnopharmacology*, *141*(3), 817-824.

3. da Silva, J. C., Barbosa, L. C. A., Demuner, A. J., Montanari, R. M., Francino, D., Meira, R. M. S. A., & de Souza, A. D. (2012). Chemical composition and histochemistry of *Sphagneticola trilobata* essential oil. *Revista Brasileira de Farmacognosia, 22*(3), 482-489.
4. Dai, Z. C., Wang, X. Y., Qi, S. S., Cai, H. H., Sun, J. F., Huang, P., & Du, D. L. (2016):Effects of leaf litter on inter-specific competitive ability of the invasive plant *Wedelia trilobata*. *Ecological Research*, *31*(3), 367–374.
5. Husain, I. N., & Kumar, A. (2015). Comparative study of phytochemical constituents in flower of *Wedelia trilobata*, *Achyranthes aspera* and *Chrysanthemum* from Durg District of Chhattisgarh. *International journal of current microbiology and applied sciences, 4*(4), 150-156.
6. Kordali, S., Cakir, A., & Sutay, S. (2007): Inhibitory effects of monoterpenes on seed germination and seedling growth. *Zeitschrift für Naturforschung C*, *62*(3-4), 207-214.
7. Nie, C., Zeng, R. S., Luo, S. M., Li, H. S., Hong, M. Q., & Cheng, L. Q. (2004): Allelopathic potentials of *Wedelia trilobata* L. on rice. *Acta Agronomica Sinica, 30*(9), 942–946.
8. Ren, H., Xu, Q. L., Luo, Y., Zhang, M., Zhou, Z. Y., Dong, L. M., & Tan, J. W. (2015). Two new ent-kaurane diterpenoids from *Wedelia trilobata* (L.) Hitchc. *Phytochemistry Letters*, *11*, 260–263.
9. Scrivanti, L. R., Zunino, M. P., & Zygadlo, J. A. (2003):*Tagetes minuta* and *Schinus areira* essential oils as allelopathic agents. *Biochemical systematics and ecology*, *31*(6), 563-572.
10. Shankar, R., & Thomas, T. (2014). Antibacterial activity of flower heads of *Wedelia trilobata* (L.) A. S. Hitchc. *Journal of Biological & Scientific Opinion*, *2*(6), 409–412.
11. Singh, H. P., Batish, D. R., Kaur, S., Arora, K., & Kohli, R. K. (2006): α-Pinene inhibits growth and induces oxidative stress in roots. *Annals of Botany*, *98*(6), 1261-1269.
12. Singh, H. P., Batish, D. R., Kaur, S., Ramezani, H., & Kohli, R. K. (2002): Comparative phytotoxicity of four monoterpenes against *Cassia occidentalis*. *Annals of Applied Biology*, *141*(2), 111-116.
13. Verma, N., & Khosa, R. L. (2015). Chemistry and biology of genus Wedelia Jacq.: A review. *Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)]*, *6*(2), 71-90.
14. Vokou, D., Douvli, P., Blionis, G. J., & Halley, J. M. (2003): Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. *Journal of chemical ecology*, *29*(10), 2281-2301.
15. Zhang, Y. H., Liu, M.F., Ling, T.J., Wei Xiao,Y.,& South, C. B. G. (2004):Allelopathic sesquiterpene lactones from *Wedelia trilobata*. *Journal of Tropical and Subtropical Botany*, *12*(6):533-537.