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Efficacy of Sesame Root Exudates against Some Major Weeds of Rabi Crops

Lalit Kumar and Jay Gopal Varshney[1]
Indian Institute of Pulse Research, Kanpur-208 024 (U. P.), India

ABSTRACT

Emulsion concentrate or emulsive water (EW) formulation developed from crystallized product of sesame root exudates showed consistent adverse effect on the germination and growth of prominent weeds found in almost all **rabi** crops. EW formulation at 240 µg/g of soil not only delayed but also inhibited germination of lambsquarters (*Chenopodium album*) by 80% followed by scarlet pimpernel (*Anagalis arvensis*) by 75%, white sweet clover (*Melilotus alba*) by 65%, corn flurry (*Spergula arvensis*) by 60%, fumitory (*Fumaria parviflora*) by 55% and common vetch (*Vicia sativa*) by 50% over control. At 280 µg/g of soil, maximum inhibition in shoot biomass was observed in case of lambsquarters (86%) followed by fumitory (82%), common vetch (52%), corn flurry (49%), scarlet pimpernel (46%) and white sweet clover (42%) over control. Whereas based on root biomass inhibition, the toxicity trend of formulation was observed on lambasquarter by 89% followed by corn flurry (83%), sweet clover (72%), common vetch (65%), fumitory (63%) and scarlet pimpernel (58%). Based on entire biomass inhibition, it was observed more toxic to lambsquarters (86%) followed by fumitory (79%), common vetch (56%), corn flurry (50%), white sweet clover (49%) and scarlet pimpernel (48%) over control at 280 µg/g concentration.

INTRODUCTION

Various workers under field conditions have recently evaluated allelopathic potential of sesame crop against purple nutsedge, which might be due to the release of secondary metabolites through roots as exudates (Chandrasekhar *et al*., 1998; Varshney, 1994). Based on these observations, we confirmed the allelopathic potential of release compounds of sesame roots, in petriplate and pot experiments against purple nutsedge. During pot experiments conducted for longer period (three months) to observe the effect of release compounds under natural conditions on *Cyprus rotundus* tuber formation we observed the deleterious effect of chemical mixture on certain other weeds too burgeon automatically in treated pots (Kumar and Varshney, 2004). Therefore, apart from *Cyprus rotundus* need was felt to confirm the allelopathic effect of release compounds on other weeds also. Considering this, a laboratory study was undertaken at IIPR, Kanpur to find out the effect of sesame allelochemicals on the germination and growth of prominent weeds, which grow in winter crops.

MATERIALS AND METHODS

Collection of Root Exudates

Root exudates from sesame crop were collected after growing the plants in root exudate trapping system comprising Buchner funnel of 110 mm diameter fitted on conical flasks of 500 ml capacity during their crop season (mid July to November last) in the years of 2003 and 2004. Sieve portion of the funnels was removed by cutting and the funnels were filled to the capacity with the soil collected from the field. Soil filled funnels were mounted on conical flasks containing distilled water and 5 to 6 germinated seeds of sesame were sown in each funnel. Out of 5-6 plants, 3 to 4 plants depending upon the growth or size of the plants were allowed to grow till maturity. After attaining the age of 15-20 days, plant roots penetrated the soil filled in funnels and emerged into the conical flasks containing distilled water. Root zone water from conical flasks was taken out regularly at an interval of 3- 4 days and replaced with fresh distilled water till maturity of sesame plants.

Isolation of Allelocompounds from Root Exudates

Allelochemicals from root exudates were isolated by passing through well-conditioned chromatographic columns (46 x 1.8 cm) packed with different types of ion exchange resin. Four chromatographic resins viz., ceralite IR 400, ceralite IRC 50, ceralite IRC 410 and ceralite IR 410 were tried for trapping the root exudates from root zone water. The technique was standardized for maximum recovery.

[1]Director, NRCW, Jabalpur.

Column that contained all the four chromatographic resins (10 g each) with one small bend of silica gel (10 g) at bottom was found suitable to trap maximum amount of allelocompounds. Sufficient numbers of columns were packed upto a height of 30 cm with these resins. Approximately 1 to 1.5 litre root zone water was passed slowly (2 ml/min) through each column. In this process, allelocompounds got absorbed on ion exchange resins. After complete elution of root zone water, allelocompounds were extracted out by eluting the columns with 500 ml of 80% methanol followed by 500 ml ethyl acetate. Methanol and ethyl acetate fractions were pulled together and evaporated to dryness under vacuum for recovery of allelocompounds.

Emulsion Concentrate

For bioassay a 10% emulsion concentrate (EW) formulation was developed by taking the required quantity (1 g) of isolated product of sesame plant. The mixture of compounds obtained so was emulsified by taking tween 80 (1 g) as emulsifier and cyclohexanone (1 g) and water (7 g) as solvents. Emulsion concentrate was obtained by vigorously agitating the mixture at 45 + 2^0C for an hour.

Preparation of Test Solutions

The test solutions of different concentrations were prepared by taking the appropriate amount of the EW and diluting it in a definite volume of water so as to get the desired concentration (40, 80, 120, 140, 160, 200, 240 and 280 µg/g) in soil filled in petri plates.

Bioassay

Laboratory bioassay was carried out during October to February of 2004 and 2005 to examine the allelopathic potentials of isolated compounds of sesame root exudates on six major weeds of pulse crops. For testing the efficacy of EW formulation on germination of test weeds the experiments were laid out in replicated petriplates containing a layer of sand of approximately 150 g moistened with 5.0 ml Hoglands nutrient solution. Twenty seeds of each test weeds viz., *Chenopodium album, Anagalis arvensis, Spergula arvensis, Melilotus alba, Fumaria parviflora* and *Vicia sativa* were sown in each Petridish. Prior to sowing, the seeds were surface sterilized with 0.1% mercuric chloride solution and the Petridish were sterilized in hot air oven at 150°C. Plates were treated with 15 ml solution of different dilutions viz., 400, 800, 1200, 1600, 2000 and 2400 µg/ml which deposited 6, 12, 18, 24, 30 and 36 mg product and produced concentrations viz., 40, 80, 120, 160, 200 and 240 µg/g of sand, respectively. In the same way, a separate set of experiment was maintained for 25 days to see the effect of allelocompounds on growth and development of test weeds. The experiment was conducted in replicated petriplates containing a layer of sand at bottom covering with filter paper. Both sand as well as filter paper was moistened with 10 ml Hogland nutrient solution. Twenty surface sterilized and germinated seeds of each test weeds were transferred in petriplates containing as per treatment 15 ml solution of different dilutions viz., 400, 800, 1200, 1600, 2000, 2400 and 2800 µg/ml prepared from 10% EW formulation. After treatment the dishes containing test weed species were kept into a controlled environmental chamber (25±2^0C, 12 h light, 12 h dark). All treatments were replicated thrice under identical conditions. A set of experiment was kept as control treated with formulation auxiliaries. Observations were recorded for germination count, radical and plumule length and their biomass after 25 days of the experimentation.

RESULTS AND DISCUSSION

Effect of EW Formulation on Germination

A great impact of assayed formulation was observed on germination of all the test weeds (Fig. 1). EW formulation not only severely inhibited the germination but also caused considerable delay in germination of all the weeds. Inhibitory and delaying effect on germination of weed seeds varied with different EW formulation concentrations. The magnitude of inhibition and delaying in germination of all the weeds increased linearly with increase in concentration. At 240 µg/g concentration EW formulation showed the maximum inhibition in germination of *C. album* (80%) followed by *A. arvensis* (75%), *M. alba* (65%), *S. arvensis* (60%), *F. parviflora* (55%) and *V. sativa* (50%) over control. At higher concentrations viz., 120 µg/g and above developed formulation caused considerable delay in germination of test weeds over control. In case of control (formulation auxiliaries viz., tween-80 and cyclohexanone) nearly 50% germination in seeds of all

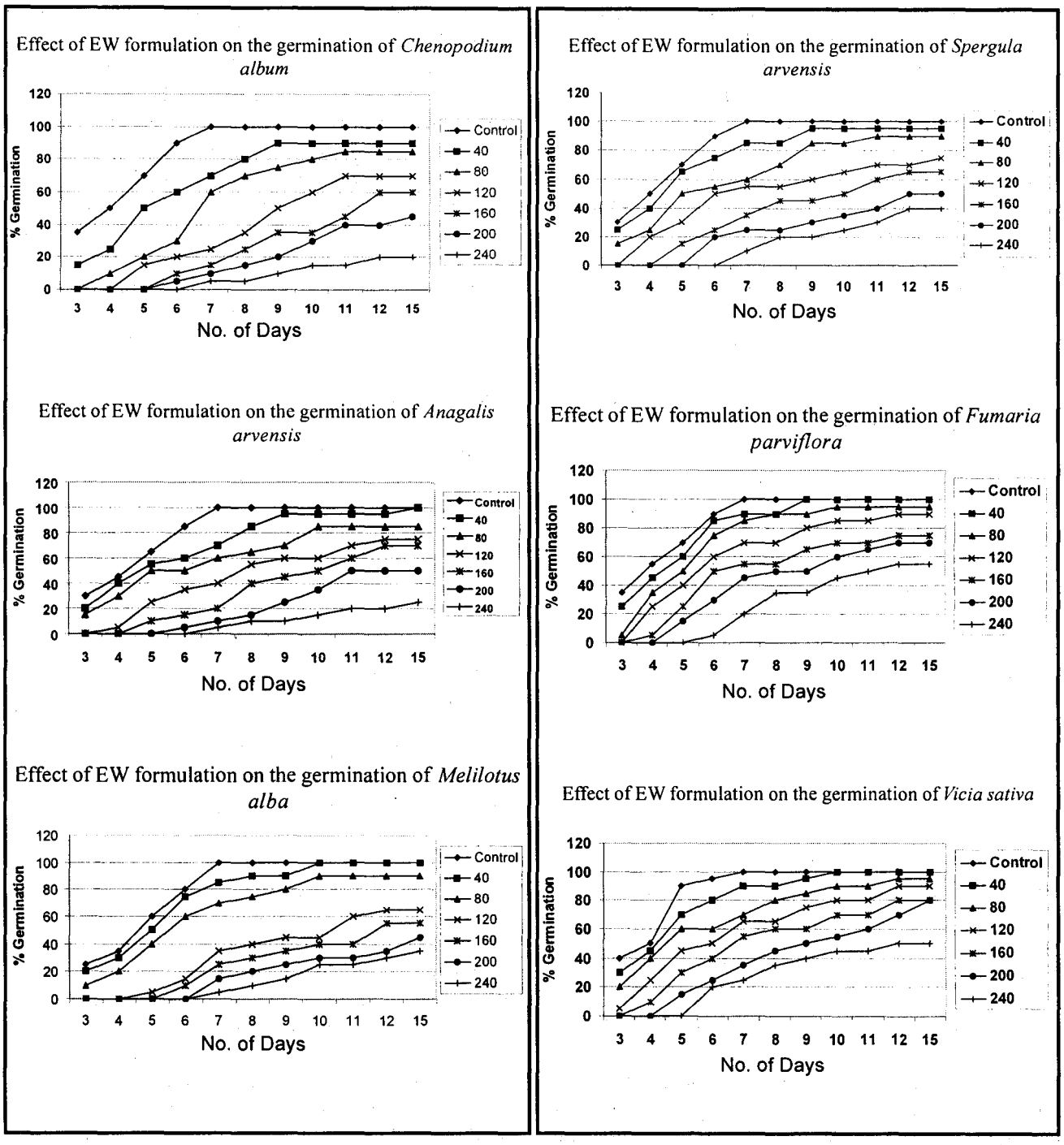

Fig. 1. Effect of EW formulation on the germination of different weeds.

Table 1. Efficacy of sesame allelochemicals against *Chenopodium album*, *Anagalis arvensis* and *Fumaria parviflora*

Parameters	Weed	Treatments								C. V.	S. Em	C. D.
		Cont.	40 µg/g	80 µg/g	120 µg/g	160 µg/g	200 µg/g	240 µg/g	280 µg/g			
Average shoot length (cm)	*C. album*	4.53	4.43	3.90	3.57	3.07	2.57	2.10	1.57	7.2	0.13	0.41
		(-)	(2.2)	(13.96)	(21.31)	(32.34)	(43.37)	(53.67)	(65.43)			
	A. arvensis	4.80	4.667	4.233	3.833	3.533	3.233	3.0	2.367	2.69	0.0576	0.247
		(-)	(2.77)	(11.81)	(20.14)	(26.39)	(32.64)	(37.5)	(50.68)			
	F. parviflora	2.80	4.467	4.167	2.033	1.867	1.600	1.400	1.067	6.07	0.067	0.204
		(-)	(11.89)	(22.6)	(27.39)	(33.32)	(42.85)	(50.0)	(61.89)			
Average root length (cm)	*C. album*	3.10	2.97	2.77	2.10	1.83	1.30	1.03	0.80	6.81	0.078	0.27
		(-)	(4.29)	(10.74)	(32.25)	(40.87)	(58.06)	(66.67)	(74.19)			
	A. arvensis	3.20	2.867	2.60	2.367	2.067	1.833	1.467	0.833	10.58	0.1309	0.396
		(-)	(10.40)	(18.75)	(26.03)	(32.28)	(42.71)	(54.15)	(73.96)			
	F. parviflora	3.233	3.933	2.60	2.233	2.0	1.867	1.50	0.933	5.61	0.07	0.21
		(-)	(9.27)	(19.57)	(30.93)	(38.13)	(42.25)	(53.6)	(71.14)			
Shoot weight weight (g)	*C. album*	0.81	0.71	0.65	0.56	0.39	0.22	0.17	0.12	6.02	0.016	0.13
		(-)	(42.22)	(19.87)	(30.37)	(51.23)	(72.83)	(79.38)	(85.80)			
	A. arvensis	0.853	0.845	0.811	0.765	0.757	0.597	0.557	0.460	5.15	0.0210	0.063
		(-)	(0.94)	(4.92)	(10.31)	(11.25)	(30.01)	(34.70)	(46.07)			
	F. parviflora	0.673	0.655	0.626	0.538	0.378	0.338	0.259	0.124	2.18	0.0057	0.017
		(-)	(2.67)	(6.98)	(20.05)	(43.83)	(49.77)	(61.51)	(81.57)			
Root weight (g)	*C. album*	0.06	0.05	0.04	0.03	0.02	0.01	0.01	0.009	7.77	0.0012	0.006
		(-)	(16.07)	(32.14)	(50.0)	(69.64)	(82.14)	(82.14)	(89.28)			
	A. arvensis	0.178	0.168	0.162	0.144	0.135	0.124	0.113	0.074	6.13	0.0049	0.015
		(-)	(5.61)	(8.98)	(19.10)	(24.15)	(30.33)	(36.51)	(58.42)			
	F. parviflora	0.134	0.125	0.117	0.100	0.090	0.075	0.061	0.050	5.30	0.0029	0.008
		(-)	(6.71)	(13.33)	(25.37)	(32.83)	(44.03)	(54.47)	(62.68)			
Total biomass (g)	*C. album*	0.866	0.758	0.687	0.592	0.412	0.230	0.176	0.121	5.88	0.0163	0.049
		(-)	(12.17)	(20.66)	(31.63)	(52.42)	(73.44)	(79.67)	(86.02)			
	A. arvensis	1.032	1.014	1.973	0.909	0.811	0.722	0.670	0.534	2.76	0.0134	0.04
		(-)	(1.74)	(5.71)	(11.91)	(18.50)	(30.0)	(35.07)	(48.25)			
	F. parviflora	0.807	1.779	1.638	0.467	0.413	0.320	0.670	0.172	2.18	0.0068	0.020
		(-)	(3.47)	(7.80)	(20.94)	(42.13)	(48.82)	(60.34)	(78.68)			

Figures in parentheses represent per cent inhibition over control.

Table 2. Efficacy of sesame allelochemicals against *Spergula arvensis*, *Vicia sativa* and *Melilotus alba*

Parameters	Weed	Treatments								C. V.	S. Em	C. D.
		Cont.	40 µg/g	80 µg/g	120 µg/g	160 µg/g	200 µg/g	240 µg/g	280 µg/g			
Average shoot length (cm)	*S. arvensis*	3.50 (-)	3.3 (5.71)	3.1 (11.42)	2.9 (17.14)	2.7 (22.85)	-	2.2 (37.15)	1.26 (64.0)	4.76	0.0745	0.22
	V. sativa	6.200 (-)	5.933 (4.30)	5.133 (17.2)	4.800 (22.58)	4.333 (30.11)	4.133 (33.33)	3.767 (39.24)	3.333 (46.24)	3.81	0.1034	0.313
	M. alba	3.700 (-)	3.333 (9.91)	3.100 (16.21)	2.900 (21.62)	2.667 (27.91)	2.500 (32.43)	2.167 (41.43)	1.933 (47.75)	5.02	0.0808	0.245
Average root length (cm)	*S. arvensis*	3.1 (-)	2.7 (12.9)	2.533 (18.29)	2.9 (25.86)	2.1 (32.25)	-	1.433 (53.77)	1.067 (65.58)	4.69	0.0589	0.178
	V. sativa	3.467 (-)	3.000 (13.5)	2.800 (19.23)	2.500 (27.89)	2.300 (33.66)	2.033 (41.36)	1.633 (52.89)	1.067 (69.22)	5.90	0.801	0.243
	M. alba	3.033 (-)	2.500 (17.6)	2.100 (30.76)	1.667 (45.03)	1.167 (51.63)	1.300 (57.138)	1.200 (60.43)	0.867 (71.41)	6.52	0.0665	0.2017
Shoot weight (g)	*S. arvensis*	0.345 (-)	0.337 (2.31)	0.312 (9.56)	0.290 (15.94)	0.224 (35.07	-	0.210 (39.13)	0.175 (49.27)	2.01	0.0031	0.009
	V. sativa	1.723 (-)	1.557 (9.63)	1.373 (20.31)	1.240 (28.03)	1.110 (35.57)	1.080 (37.31)	0.970 (43.70)	0.820 (52.40)	3.79	0.027	0.082
	M. alba	0.124 (-)	0.117 (5.65)	0.108 (12.90)	0.103 (10.93)	0.096 (22.56)	1.088 (29.03)	0.080 (35.46)	0.072 (41.93)	14.68	0.0086	0.026
Root weight (g)	*S. arvensis*	0.012 (-)	0.011 (8.33)	0.011 (8.33)	0.009 (25)	0.006 (50)	-	0.003 (75)	0.002 (83.33)	17.89	0.0009	0.0027
	V. sativa	0.806 (-)	0.731 (9.30)	0.683 (15.26)	0.646 (19.85)	0.620 (23.07)	0.600 (25.55)	0.445 (44.78)	0.286 (64.51)	2.10	0.0073	0.022
	M. alba	0.036 (-)	0.031 (13.9)	0.025 (30.55)	0.017 (52.77)	0.014 (61.11)	0.012 (66.66)	0.010 (72.22)	0.010 (72.22)	9.66	0.0011	0.003
Total biomass (g)	*S. arvensis*	0.357 (-)	0.354 (0.84)	0.322 (9.80)	0.300 (15.96)	0.230 (35.57)	-	0.213 (40.33)	0.177 (50.42)	2.0	0.0032	0.009
	V. sativa	2.529 (-)	2.228 (9.25)	2.057 (18.66)	1.886 (25.42)	1.769 (30.05)	1.680 (33.57)	1.415 (44.05)	1.106 (56.26)	2.75	0.0292	0.088
	M. alba	0.161 (-)	0.148 (8.07)	0.133 (17.39)	0.119 (23.60)	0.110 (31.67)	0.101 (37.26)	0.090 (44.09)	0.082 (49.07)	3.91	0.0027	0.008

Figures in parentheses represent per cent inhibition over control.

the weeds were observed on 4th day of planting and 100% germination was achieved within one week, whereas in case of various treatments viz., 40, 80, 120, 160, 200 and 240 µg/g 100% germination could not be achieved even after 15 days of transplanting. At higher concentrations i. e. 160, 200 and 240 µg/g first sprouting could be seen only after one week of sowing. The effect was found almost at par in the germination of entire weed species tested.

Effect of EW Formulation on Shoot Growth

The effect of assayed formulation on shoot growth of weeds was observed in different weed flora (Tables 1 and 2). Results revealed a significant reduction in shoot length and shoot biomass of test weeds. The magnitude of reduction was found concentration dependent i. e. increase in concentration of EW formulation was associated with drastic inhibition in shoot growth and shoot biomass of all the test weeds. Approximately 2-10% and 5-15% inhibition in shoot length and shoot biomass of test weeds was observed at lowest concentration (40 µg/g) over control. Effect of formulation at subsequent increasing concentrations increased linearly hence a good correspondence between concentration of formulation and its effect was observed. Maximum inhibition in both shoot length and shoot biomass was observed at highest concentration (280 µg/g) over control. Depending upon the test weed species the magnitude of inhibition based on shoot length and shoot biomass varied due to wide genetic variability in their canopies. Therefore, no correlation among the weeds between inhibition based on shoot length and shoot biomass with formulation concentrations was observed. The magnitude of inhibition as per shoot length at 280 µg/g concentration followed the order : *C. album* (65%), *S. arvensis* (64%), *F. parviflora* (62%), *A. arvensis* (51%), *M. alba* (48%) and *V. sativa* (46%), whereas the magnitude of inhibition as per shoot biomass followed the order : *C. album* (86%), *F. parviflora* (82%), *V. sativa* (52%), *S. arvensis* (49%), *A. arvensis* (46%) and *M. alba* (42%).

Effect of EW Formulation on Root Growth

Predominant effect of assayed EW formulation was observed on growth of radicals of all the test weeds, hence root length and root biomasses were taken as most affected parameters for assessing the efficacy of assayed formulation. In case of root length and root biomass also a good correspondence between concentration and effect was observed (Tables 1 and 2). Approximately 10-15% inhibition in both i. e. root length and biomass of test weeds was observed at lowest concentration i. e. 40 mg/g over control. At subsequent increasing concentrations the inhibition in root length and root biomass was increased linearly. At 280 µg/g concentration it drastically inhibited the growth of radicals of the entire weed species tested. At same concentration prepared formulation was found more toxic to *C. album* by inhibiting the root length and root biomass by 74 and 89%, respectively. The magnitude of inhibition in radical length of remaining weeds at same concentration of the formulation followed the order : *A. arvensis* (74%), *M. alba* (71%), *F. parviflora* (71%), *V. sativa* (69%) and *S. arvensis* (66%). The formulation concentration above 280 µg/g inhibited completely the root development of all the test weeds. Since the roots of all the six weeds varied genotypically in their rhizosphere hence no correlation between inhibitions based on root length and root biomass with a particular concentration of formulation in different weeds was observed. Therefore, based on root biomass inhibition the toxicity of developed formulation followed the order of *C. album* (89%), *S. arvensis* (83%), *M. alba* (72%) *V. sativa* (65%), *F. parviflora* (63%) and *A. arvensis* (58%). For assessing the toxicity of developed formulation in totality entire biomass of weeds can be taken as affected parameter. Based on entire biomass inhibition, the formulation was observed more toxic to *C. album* (86%) followed by *F. parviflora* (79%), *V. sativa* (56%), *S. arvensis* (50%), *M. alba* (49%) and *A. arvensis* (48%).

Apart from the inhibitory effect on root length and root biomass, toxicity symptoms were also observed on root tips. In severe phytotoxicity i. e. at higher concentrations, the root tips of seedling roots of all the test weeds turned dark brown, stopped growing and decomposing completely after 4-5 days of treatment. In weak toxicity, seedling roots continue to grow but were short and their lower part began to decay. Due to the decomposition in roots wilting symptoms in green leaves of some of the plants were also observed. The results are in good agreement with the previous study carried out by us in which the same formulation caused complete degradation in the roots of *C. rotundus* and ultimately led to 100% mortality in plants after two months of treatments. Though the allelopathic study of sesame root exudates, in our laboratory, is being taken exclusively

for control of *C. rotundus* but the good control of other weeds too indicated the presence of some wide spectrum allelomolecules in sesame root exudates. Wide spectrum activity of sesame root exudates could be either due to the presence of chemically different groups of molecules or non selective action of molecules if belonging to identical group which needs to be confirmed. Therefore, the allelochemicals belonging to sesame root exudates could be good candidate for the development of new herbicidal model for weed control in pulses and other crops. Fractionation of entire collected root exudates of sesame into individual compounds and characterization of fractionated compounds by both bioassay (weeds and crops) as well as structure elucidation is needed to confirm this hypothesis. The work is presently going on in this line in our laboratory.

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