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# Allelopathic effect of sesame root exudates against purple nut sedge

**Lalit Kumar and Jay G. Varshney[1]**
*Division of Crop Production, Indian Institute of Pulses Research, Kalyanpur, Kanpur (Uttar Pradesh)*
*[1]National Research Centre for Weed Science, Adhartal, Jabalpur (Madhya Pradesh)*
*Email : lalit1997@ymail.com*

**ABSTRACT**

**Emulsive water concentrate (EW) formulation of allelocompounds obtained from root exudates of sesame plants applied on tubers of purple nut sedge caused significant delay and inhibition in germination. EW formulation at 240 µg/g concentration of soil inhibited shoots and root biomass by 81.3 and 91.9%, respectively over control. Application of unformulated product on 15 days germinated tubers caused no significant effect on their growth and development at varying concentrations (0-240 µg/g soil) but showed adverse effect after some time. In pot experiment, isolated root exudates caused severe reduction in total shoot and root biomass of purple nut sedge. After two months, maximum reduction in total biomass and root biomass was observed by 68.0 and 90.0%, respectively at 240 µg/g of soil. Application of 240 µg/g of purified allelocompounds of root exudates caused 97% reduction in total number of newly formed tubers.**

**Key words :** Allelocompounds, Nut sedge, Root exudates

Purple nut sedge (*Cyperus rotundus* L.) is one of the most troublesome perennial weed of field crops found in tropics and subtropics throughout the world and cause considerable reduction in crop yields. It propagates mainly through tubers. The weed is managed either by manual weeding or by use of herbicide. The former method is costly, time consuming and not feasible for large area while the later method is cheap, creates soil and water pollution and needs technical knowhow for its application. To overcome these problems, biological control appears to be economical option for control of such weeds. In recent years, allelopathic suppression of weeds is receiving greater attention (Duke 1986, Duke and Lydon 1987, Duke *et al.* 2000). Some of the secondary metabolites isolated from different plant species have been found to be active against various weeds and some of them are being used as natural herbicides for control of different weeds (Shiling *et al.* 1986, Lydon *et al.* 1989, Yu and Matsui 1994). Allelopathic effect of sesame crop against purple nut sedge has been evaluated by various workers (Chandrasekhar *et al.* 1998, Nesser and Varshney 2001, Varshney 1993,1994). Suppression of purple nut sedge in sesame field may be due to the release of secondary metabolites through root as exudates. No effort has so far been made to assess the allelopathic potential of sesame root exudates released by intact live plants during their life cycle. Allelochemicals in sesame root exudates can thus be exploited as natural herbicides or as leads for development of more potent herbicide against this obnoxious weed. This study attempts to extranet the root exudates of sesame plant and to assess its effect against purple nut sedge.

## MATERIALS AND METHODS

### Collection of root exudates

Root exudates from sesame (*Sesamum indicum*) were collected by the procedure as suggested by Kumar and Varshney (2004), after growing the plants in root exudates trapping system comprising of Buchner funnel of 110 mm dia fitted on conical flasks of 500 ml capacity. Central holes and few other holes of the sieve of the funnels were removed by cutting and the funnels were filled to the capacity with the soil collected from the field. Muslin cloth piece was used for holding the soil in funnel. Soil filled funnel was mounted on conical flask containing distilled water. Five to six germinated seeds of sesame were sown in each funnel. Out of five-six plants, three to four plants depending upon the growth or size of the plants were allowed to grow till maturity. After attaining the age of 15 to 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**

The root exudates in a aqueous solution were isolated by passing through well-conditioned chromatographic columns (46 x 1.8 cm) packed with different type of ion exchange resins. Four chromatographic resins *viz.*, ceralite IR 410, ceralite IR 400, ceralite IRC 50 and ceralite IRC 410 were tried for trapping the root exudates from root zone water. The technique was standardized for maximum recovery. Column that contained all the four chromatographic resins (10g each) with one small band of silica gel (10g) at bottom was found suitable to trap maximum amount of allelocompounds. Sufficient number of columns were packed up to an height of 30 cm with these resins. Approximately 1 to 1½ litre root zone water was passed slowly (2ml/minute) 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 pooled together and evaporated to dryness under vacuum for recovery of allelocompounds.

**Emulsion concentrate formulation**

For bioassay, a 10% emulsion water concentrate (EW) formulation was developed by taking the required quantity (1g) of exudates extractedfrom sesame plant. The exudates having mixture of compounds obtained so was emulsified by taking The Tween-80 as emulsifier (1g) and cyclohexanone (1g) and water (7g). Emulsion concentrate was obtained by vigorously agitating the mixture at 45 $\pm$ $2^0$C for an hour.

**Preparation of test solutions**

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

**Bioassay**

Both formulated (10% EW) and unformulated materials (isolated allelocompounds mixture) of sesame were assessed for allelopathic potential against purple nut sedge (*Cyperus rotundus)*. For testing the efficacy of prepared EW formulation against purple nut sedge, an experiment was laid out in petriplates (90 mm dia) filled to the capacity (150 g) with normal soil from farm field. Five fresh tubers of uniform size of nut sedge were placed in each plate. Petriplates were treated with solution of formulations to get concentrations of 40, 80, 120, 160, 200, and 240 µg/g soil. The uncovered dishes containing test tubers were kept into a controlled environmental chamber (25 $\pm$ $2^0$C, 12 h light, 12 h dark). All treatments were replicated three times under identical condition. A set of experiment was kept as control with solution of formulation auxiliaries (Tween-80 + cyclohexanone + water). Observations were recorded daily on per cent germination against control.

In another experiment, 7 days old germinated tubers were treated with different concentrations of exudate *viz.*, 250, 500, 750, 1000, 1250 and 1500 µg/ml made by taking the isolated product. Required dilutions were prepared by dissolving the required quantity of product in appropriate quantity of water. In this experiment, soil was replaced with a disc of germination paper in the petridish. Initially the germination papers were moistened with 5.0 ml Hogland's solution. Ten surface sterilized (with 0.1% mercuric chloride solution) germinated tubers were placed in each Petridish. The tubers were treated thrice (at the interval of 15 days) by taking each time 10 ml solution of each concentration. Treatments were replicated three times and the experiment was maintained for two months. Control was also set up using tap water only. Moisture in the petridish was maintained by adding tap water regularly.

Small pot experiment was also conducted to confirm the bioefficacy of exudate under natural environment and to see the effect of exudate on tuber formation and their biomass. Pots were filled to the capacity (1.25 kg) with the normal soil collected from IIPR farm . Three uniform size tubers were sown in each pot. Pots were treated thrice at an interval of 15 days with the EW formulation of allelocompounds to get a concentration of 40, 80, 120, 160, 200, and 240 µg/g soils. Experiments were maintained for two months at ambient conditions. During the period, sufficient moisture was maintained in the pots by applying necessary water as and when required. Experiment was maintained in four replications along with an untreated set of control. After two months of treatment, plants were taken out from the pots by exposing the pots under running water tap and the observations were taken on root and shoot biomass, total biomass, tuber formation and newly formed tuber biomass.

## RESULTS AND DISCUSSION

**Extraction of exudate**

All the chromatographic resins *viz.*, ceralite IR 410, ceralite IRC 50 and ceralite IR 400 were found suitable for trapping the allelochemicals from collected root zone water. Maximum recovery was obtained from the columns containing the mixture of all the three ceralites (65 mg) compared to the columns containing ceralite IR 410 (50 mg), ceralite IRC 50 (30 mg) and ceralite IR 400 (45 mg). During this experiment, 15 gram product was isolated from 20 sets of root exudate trapping system of 50 to 60 plants of sesame during the crop seasons.

**Effect of EW formulation on germination**

A considerable impact of emulsion concentrate formulation prepared from isolated sesame root exudates was observed on the germination of purple nut sedge (Table 1). EW formulation not only severely inhibited the germination but also caused considerable delay in germination of purple nut sedge. Nearly 40% inhibition in germination of purple nut sedge was observed in 80, 120 and 240 µg allelocompound per g of soil over control. Though the magnitude of inhibition was not found directly proportional to concentration but increase in concentration was directly associated with delay in the germination of purple nut sedge tubers. In case of control, nearly 30% tubers were germinated at 3rd day of planting and 100% germination was achieved on 7th day of planting, whereas in case of various treatments 100% germination could not be achieved even after 15 days of planting. At higher concentrations (200 and 240 µg/g of soil), emergence of first plant could be seen only after one week of transplanting. About 50% germination was achieved at 4th, 6th, 7th and 10th days of planting in concentrations having control, 40 and 80, 120 and 160, 200 and 240 µg/g of soil, respectively. A severe inhibition in root and shoot biomass of germinated plants was also observed. EW formulation severely inhibited the shoot and root biomass by 10-80 and 16-92%, respectively at various concentrations tested, over control (Table 2).

**Bioassay of unformulated product**

To evaluate the effect and target site of extracted compound of sesame root exudates, bioassay was done in replicated petriplates by treating the tubers with 6 different concentrations *viz.* 250, 500, 750, 1000, 1250 and 1500 µg/ml of isolated product prepared by dissolving the appropriate quantity of compound in distilled water. The effect was observed on 7 days old germinated tubers and experiment was maintained for two months. At initial stage (up to 15 days), no significant influence of allelochemical was observed on growth and development of purple nut sedge in any treatments, but after one month degradation started in the roots of treated plants. Hence, suppression in root and shoot of treated plants become visible. Toxicity symptoms at root tips of germinated tubers of purple nut sedge were observed initially. In severe phytotoxicity, i.e. at higher concentrations, root tips of germinated plants turned dark brown, stopped growing and started decomposing. After one and half month, the damage to the major roots continued but was less as compared to control. After two months, the root of treated plants degraded completely in all the treatments, consequently plants died completely. Germination papers also turned completely black in all the treatments which may be due to the influence of biomolecules produced during decomposition of root in allelochemical treated nut sedge plants, whereas, in case of control the papers remained unaffected. Though the ultimate result remained unaffected with concentrations but the magnitude of effect was found more pronounced in higher concentrations. Based on this experiment, it is presumed that allelochemicals released naturally from sesame root as exudates have greater ability to suppress and degrade the roots of purple

**Table 1. Effect of EW formulation of sesame root exudate on germination (N=15) of purple nutsedge.**

| Concentration of exudate (µg/g soil) | Germination count at different days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th | 11th | 12th | 15th |
| Control | 5 | 7 | 11 | 14 | 15 | - | - | - | - | - | 15 |
| 40 | 2 | 4 | 5 | 6 | 9 | 11 | 14 | - | - | - | 4 |
| 80 | 1 | 3 | 5 | 8 | 8 | 9 | - | - | - | - | 9 |
| 120 | - | 2 | 2 | 5 | 7 | 7 | 8 | 8 | 9 | - | 9 |
| 160 | - | - | 3 | 6 | 8 | 8 | 9 | 9 | 10 | 11 | 11 |
| 200 | - | - | - | 1 | 4 | 4 | 6 | 7 | 8 | 11 | 11 |
| 240 | - | - | - | - | 2 | 5 | 8 | 8 | 8 | 9 | 9 |

**Table 2. Effect of EW formulation on growth and biomass of purple nutsedge**

| Concentration of exudate (µg/g soil) | % Inhibition in germination | Average shoot length (cm) | Shoot biomass (g) | Root biomass (g) |
|---|---|---|---|---|
| Control | 100 (00.0) | 8.2 (00.00) | 1.6 (00.0) | 1.3 (00.0) |
| 40 | 100-93.33 (06.7) | 7.2 (12.19) | 1.4 (12.5) | 1.1 (15.4) |
| 80 | 100-60.00 (40.0) | 6.3 (23.02) | 1.1 (31.2) | 0.8 (38.5) |
| 120 | 100-60.00 (40.0) | 5.2 (36.06) | 0.8 (50.0) | 0.5 (61.5) |
| 160 | 100-73.33 (26.7) | 3.3 (60.00) | 0.8 (50.0) | 0.4 (69.2) |
| 200 | 100-73.33 (26.7) | 2.4 (70.07) | 0.6 (62.5) | 0.3 (78.0) |
| 240 | 100-60.00 (40.0) | 0.9 (89.00) | 0.3 (81.3) | 0.1 (92.3) |

* Figures in parenthesis indicate % reduction in biomass.

**Table 3. Effect of sesame root exudates on purple nutsedge tuber formation.**

| Concentration of allelocompound (µg/g soil) | Shoot biomass (g) | Root biomass (g) | Density of newly formed tubers | Biomass of newly formed tubers (g) |
|---|---|---|---|---|
| Control | 15.7 (0.0) | 6.7 (0.0) | 35 (0.0) | 4.0 (0.0) |
| 40 | 10.6 (32.5) | 3.3 (50.7) | 24 (31.4) | 2.0 (50.0) |
| 80 | 9.8 (37.6) | 2.8 (58.2) | 19 (45.7) | 1.9 (52.5) |
| 120 | 7.7 (51.0) | 2.2 (67.2) | 16 (54.3) | 1.1 (72.5) |
| 160 | 7.5 (52.2) | 1.5 (77.6) | 7 (80.0) | 0.6 (85.0) |
| 200 | 5.2 (67.0) | 1.5 (77.6) | 5 (85.7) | 0.7 (82.5) |
| 240 | 5.0 (68.1) | 0.7 (89.6) | 1 (97.1) | 0.1 (97.5) |

* Figures in parenthesis indicate % reduction.

nut sedge which would likely affect the reproductive potential of the weed, because it propagates in field by continuously forming the tubers on roots. Hence, the results confirmed the presence of some very active unknown molecules in sesame root exudates, which inhibit the growth of nut sedge by degrading its roots and ultimately suppress the weed population in field. Comparatively higher activity of product in its formulation (EW formulation) might be due to the influence of formulation auxiliary's namely cyclohexanone and tween-80 that can enhance the efficacy of active ingredient.

**Effect of exudate compounds on tuber formation**

Both the petridish experiments indicated the predominant impact of exudate on roots of germinated tubers, whereas the pot experiments indicated the effect of extracted compounds on tuber formation and their biomass under natural condition. This can be taken as most effective parameter for control of purple nut sedge because unlike other weeds, the control of nut sedge is somewhat difficult owing to its tuber generation capacity. Tubers are mainly present 10 to 30 cm below in soil, hence require very specific and systematic approach to control this weed. Therefore, it was felt to confirm the activity of exudate on purple nut sedge tubers under natural conditions. Results (Table 3) revealed reduction in shoot biomass by 32.6 to 68.1 and root biomass by 50.7 to 89.6 per cent. The effect of exudate was found more on tuber formation and their biomass. About 97% reduction in total number of newly formed tubers and their biomass was observed at higher dose i.e. 240 µg/g of soil over control. Though the impact of chemical in pot experiments at these doses and duration was found concentration dependent but considerable reduction in root and tuber biomass (50%) at lowest dose i.e. 40 µg/g soils indicated the prominence of time factor over dose. Bioefficacy data strongly suggested that extracted exudate of sesame possess potential herbicidal activity against purple nut sedge in all respects. The allelocompounds not only delayed and inhibited the germination of purple nut sedge but completely destroyed the plants by degrading their roots which lead to break the cycle of propagation of this weed. Data also highlighted the role of formulation auxiliaries in influencing the activity of isolated products of sesame root exudates. Exudate in EW formulation not only showed activity at low doses but also took comparatively less time to exhibit the effect. This result suggests the presence of promising bio-active molcule in sesame root exudate. Fractionation of entire collected root exudates of sesame into individual compounds and characterization of fractionated compounds by both bioassays as well as structure elucidation is needed to confirm this hypothesis.

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