Allelopathic potential and allelochemicals in different intercrops for weed management in rainfed cotton

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Allelochemicals released by plants serve as the primary defence by targeting the establishment of weeds and other plants. In this study, 12 different intercrops were assessed over five seasons for total phenol and terpenoid content. A detailed analysis on allelochemicals produced was also done using gas chromatography–mass spectrometry (GC–MS) to correlate with their weed suppression efficiency. Total phenol content of intercrops ranged from 6.5 to 17.6 mg g^{-1} tissue dry wt, with the highest value in carom followed by sorghum, sunnhemp and marigold. Total terpenoid content of leaf extracts of the intercrops varied from 14.5 to 35.9 μg g^{-1} tissue dry wt, wherein pearl millet had maximum terpenoid content (35.9 μg g^{-1} tissue dry wt) followed by sunnhemp and sesame. Analysis using GC–MS indicated the presence of some unique as well as common allelochemicals in the experimental intercrops. To correlate the abundance of these allelochemicals released from intercrops with their weed suppression competence, relative neighbour effect (RNE) value was determined for each intercrop. Positive RNE values for sunnhemp, pearl millet and sesame indicate their efficiency in effectively reducing weed population than the other intercrops. Thus, intercrops with high phenolic, terpenoid and other allelochemicals specific to sunnhemp, pearl millet and sesame can be correlated well with weed suppression as perceptible from their RNE values.

Keywords: Allelochemicals, allelopathic potential, intercrops, rainfed cotton, relative neighbour effect.

THE defence system of plants includes constitutive as well as inducible defence as a response against herbivores and pathogens[1,2], which further involves an array of primary and secondary metabolites. Though labelled as secondary in terms of their involvement in plant metabolism, these metabolites serve as the first line of defence for plants to suppress competition, being primarily involved in the inducible defence mechanism[3]. Allelopathy, a subdiscipline of chemical ecology, is an approach to influence the growth, development, and distribution of other plants in nature utilizing such secondary substances through different mechanisms. Plants can produce and/or accumulate these bioactive metabolites in almost all parts and tissues, such as leaves, roots, stems, rhizomes, flowers, fruits and seeds[4,5].

Several studies have been conducted earlier for weed control using intercrops in different cropping systems. Various cover crops have been evaluated for their allelopathic role in weed suppression during the last decade[6–8]. Studies revealed that the extracts from legume and cereal grain crops inhibited the radicle elongation in cotton (*Gossypium hirsutum* L.) as well as in radish during different bioassays[7]. In another study, sorgoleone released from sorghum (*Sorghum bicolor* (L.) Moench) effectively controlled weeds in irrigated cotton[9]. When grown as intercrop, aromatic crops such as fenugreek (*Trigonella foenum-graecum* L.) provide excellent weed control[10]. Intercropping with crops that produce allelochemicals such as sunflower and brassicas has also been tested for weed control in cotton and other cropping systems[11,12]. However, this approach remains less popular due to lack of knowledge about the allelopathic potential of such crops, complexity in their mode of action as well as allelopathy in common. To offer intercropping as a viable option for weed control, the allelopathic potential and likely mechanisms of weed suppression by intercrops must be deciphered. Studies were conducted by growing various cover crops in the inter-row spaces of rainfed cotton (*G. hirsutum*) on Vertisols. The main objective was to identify the allelochemicals contributing to weed suppression by quantifying the relative neighbour effect (RNE).

Materials and methods

Location and intercrop details

All the field experiments were conducted at ICAR-Central Institute for Cotton Research (CICR), Panjari Farm, south of Nagpur (21°04′48.39″N, 78°06′58.02″E) in Central India. Twelve different intercrops, namely bitter cumin (*Centratherum anthelminticum* (L.) Kuntz), carom seed (*Trachyspermum ammi* (L.) Sprague, coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* Mill.), fenugreek (*T. foenum-graecum*), oats .(*Avena sativa* L.), pearl millet (*Pennisetum glaucum* (L). R. Br.), sorghum (*S. bicolor*), marigold (*Tagetes erecta*

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L.), sesame (*Sesamum indicum* L.), natively occurring mixed species (*Desmodium triflorum* (L.) DC., *Desmodium dichotomum* (Willd.) DC. and *Codariocalyx motorius* (Houtt.) H. Ohashi), and sunnhemp (*Crotalaria juncea*) were sown 30 days after cotton sowing (DACS), except marigold and oats, in inter-row spaces of cotton on Vertisols. Marigold was raised in a nursery and 10-day-old seedlings were transplanted while oats, a winter season crop, was sown in September.

Estimation of phenol content

The total phenolic content (TPC) of leaf extracts was determined according to the method described by Mallick and Singh[13]. For this, 0.25 g of leaf sample was extracted in 5 ml of 80% ethanol and boiled for 10 min to avoid oxidation of phenol by phenol oxidase. Samples were then cooled and macerated with 80% ethanol to make the final volume up to 5 ml. Then they were centrifuged for 5 min and 1 ml supernatant was taken for analysis. To this, 1 ml of Folin reagent and 1 ml 20% sodium carbonate were added, making its final volume to 10 ml with water. Absorbance was then read at 650 nm. Catechol was used to prepare standards, ranging from 200 to 1000 ppm.

Estimation of total terpenoid

Total terpenoid content was estimated following the method of Ghorai *et al.*[14]. For this, 0.5 g of tissue was homogenized with 3.5 ml of ice-cold 95% methanol (vol./vol.) in an ice-cold mortar and pestle. Samples were centrifuged at 4000 *g* for 15 min at room temperature and the supernatant was collected. To 200 μl of the supernatant, 1.5 ml chloroform was added. It was vortexed and allowed to stand for 3 min. Further, 100 μl concentrated sulphuric acid (H_2SO_4) was added to each tube by placing them on an ice pad. Then, the assay tubes were incubated at room temperature for 1.5–2 h in the dark. At the end of incubation, the supernatant reaction mixture was decanted without disturbing the reddish-brown precipitation. To the precipitate, 1.5 ml of 95% (vol./vol.) methanol was added and vortexed thoroughly until all the precipitation dissolved completely. The dissolved assay solution was then read at 538 nm. Linalool was used as the standard and total terpenoid concentration of unknown plant sample was calculated as linalool equivalents using the regression equation of linalool standard curve.

Gas chromatography–mass spectrometry analysis

Samples were prepared from the leaves of different cover crops using HPLC-grade methanol (Sigma-Aldrich®, USA) in the ratio 1 : 10. The leaf extract was then subjected to a combined gas chromatography–mass spectrometry (GC–MS) system (Shimadzu QP2020) for identification of allelochemicals. The non-polar 1,4-bis(dimethylsiloxy) phenylene dimethyl polysiloxane capillary column (Rxi-5 Sil MS) with maximum temperature of 320°C (0.25 mm × 30 m × 0.25 μm) was used for the separation of fractions. For removal of contaminants, mobile phase helium with 99.999% purity (LabPulse India Ltd, Mumbai) was passed through the universal trap.

A splitless inlet was used with inlet temperature of 280°C. The oven temperature was maintained initially at 40°C min^{-1} with 3 min hold and a ramp of 10°C min^{-1} till 250°C and then held for 25 min with column (Rxi-5 Sil MS) flow of 1.4 ml/min with linear velocity of 38.6 cm/sec and pressure of 60 kPa. Sample was injected into the column in 1 μl aliquots. The temperature of the ion source was maintained at 200°C. For identification and quantification of the compounds, data were evaluated by TIC (total ion count) and the mass spectra generated using MS were compared with the stored database of NIST mass spectral library (NIST 2014 version).

Relative neighbour effect

Relative neighbour effect (RNE) value was calculated to assess the performance of an intercrop in checking the weed flora as follows[15]

$$\text{RNE} = ((N_{\text{wic}} - N_{\text{ic}}))/x, \quad (1)$$

where N_{wic} is the weed density in the treatment without an intercrop, the weedy check, and N_{ic} is weed density in the intercrop plots. Weed density (x) depends on the treatment having greater weed density. If $N_{\text{wic}} > N_{\text{ic}}$, then $x = N_{\text{wic}}$ and vice versa.

Results

Total phenol and terpenoid content of different intercrops

TPC of the above-ground biomass of intercrops varied from 6.5 to 20.6 mg g^{-1}, with the highest value observed in carom seed followed by the sorghum, marigold and sunnhemp intercrops (Table 1). Oats was observed to have the lowest phenolic content (6.5 mg g^{-1}) among the intercrops tested.

Terpenoid content was lower than TPC in all the intercrops with a range 14.5–35.9 μg g^{-1} in different intercrops. Pearl millet had the highest value of terpenoid content and it was 1.2–2.5-fold greater than the other intercrops. The terpenoid content of pearl millet, carom seed, sunnhemp and sesame were nearly identical (32.5–35.9 μg g^{-1} linalool equivalents), whereas desmodium had the lowest terpenoid content when compared to the other intercrops.

Allelochemicals in intercrops

The chemical constituents differed in the intercrops belonging to various categories, such as aliphatic acids, aldehydes, sterols, etc. Though the abundance varied, some intercrops had few similar constituents along with other unique compounds (Table 2). For instance, among the various chemical compounds, phytol and 9,12,15-octadecatrienoic acid was present in six, neophytadiene in five, dotriacontane in four and γ-sitosterol in three of the 12 intercrops. Marigold had the maximum number of compounds with differences in their relative abundance, followed by oats and fenugreek. The list of the compounds with their relative abundance in intercrops under investigation is provided in the Supplementary Table 1. The chromatograms and relative abundance of different compounds in intercrops are also provided in the Supplementary Figure 1.

Relative neighbour effect of intercrops

RNE was assessed to identify the effectiveness of an intercrop to suppress the weeds. Table 3 shows that all the intercrop treatments reduced weed emergence when compared to the control plot. Mean values of RNE were the greatest for the sunnhemp intercrop treatment followed by the pearl millet and sesame treatments. The remaining intercrop treatments had values ranging from –0.25 to –0.53. However, compared to the Farmers' Practice (FP) treatment, four intercrop treatments, viz. sunnhemp, pearl millet sesame and sorghum suppressed the weeds, whereas all the other intercrop treatments did not suppress weed emergence having values ranging from –0.04 to –0.21.

Discussion

The plants secondary metabolites, mainly phenols and terpenoids, have been explored considerably for their utilization as natural herbicides. Studies on phytotoxicity have confirmed the role of phenolic and terpenoid compounds in imparting inhibitory action on weed germination and weed growth. In the present study, TPC and terpenoid content of intercrops ranged from 6.5 to 17.6 μg g^{-1} and 14.5 to 35.9 μg g^{-1} tissue dry wt respectively. Among different cover crops assessed, carom had the highest content of phenolic compounds followed by sorghum, marigold and sunnhemp. Higher concentration of different phenolic compounds has been reported to have better weed control efficiency with additive effect when used in combination[16]. Though carom had the highest TPC, weed suppression effect was not consistent over the years. The pattern of release and mode of action of phenolic compounds vary and depend on soil moisture and climatic conditions, apart from being dependent on the crops[17,18]. This could have been attributed to inconsistent weed control efficiency of carom[19]. Also, some plants reduce the effects of allelochemicals produced by neighbouring plants utilizing detoxifying mechanisms like conjugation, sequestration or secretion of carbohydrates, and oxidation of phytotoxic compounds[20]. In few cases, rhizosphere microorganisms may decrease the phytotoxicity of allelopathic phenolic compounds by mineralization and/or sorption and oxidation, thus diminishing the phytotoxicity of phenolic components[21]. On the other hand, effective weed control in sunnhemp could be due to fast growth, and high TPC and terpenoid content. Natural compounds such as phenols and terpenes (monoterpenes, phenylpropenes and sesquiterpenes) were found to act as bioherbicides against *Echinochloa crus-galli* under laboratory and glasshouse conditions[22] by way of disruption of metabolic enzymes involved in glycolysis and oxidative pentose phosphate pathway[23].

Thus, the higher weed suppression effect of sunnhemp and pearl millet could be attributed to the high TPC and terpenoid content, which can inhibit seed germination and seedling growth. Furthermore, terpenoids affect the growth of weeds by inhibiting adenosine triphosphate (ATP) formation, disrupting the hormonal activity, by forming a complex with proteins, and/or by inhibition of respiration[24]. In the present study, relative abundance of squalene was found to be higher in pearl millet, which is known to inhibit the germination of seeds of the epiphyte *Tillandsia recurvata*[25]. Here, intercrops with high terpenoid content (sunnhemp and pearl millet) showed better weed control compared to others, establishing the direct proportionality of terpenoid content of intercrops with their allelopathic potential.

RNE value was determined to assess weed suppression by an intercrop and its mulch. Positive RNE values ranging from 0 to 1 indicate weed suppression and negative values (0 to –1) indicate weed facilitation by the intercrop[26]. Positive RNE values compared to the control treatment clearly indicate weed suppression. However, a farmer is never going to maintain his field in a weedy

Table 1. Average phenol and terpenoid content (per g tissue dry wt) of different intercrops

Intercrops	Total phenol (mg/g)	Total terpenoids (μg/g)
Bitter cumin	14.4 ± 2.9	28.8 ± 7.4
Carom	17.6 ± 2.0	32.5 ± 5.7
Coriander	8.4 ± 2.1	28.9 ± 12.3
Fennel	9.1 ± 1.0	21.2 ± 2.7
Fenugreek	9.4 ± 4.3	29.3 ± 7.6
Oats	6.5 ± 2.1	22.6 ± 2.0
Pearl millet	10.6 ± 1.2	35.9 ± 2.1
Sorghum	15.4 ± 5.2	27.0 ± 4.0
Desmodium	13.0 ± 3.9	14.5 ± 5.1
Sunnhemp	14.6 ± 4.2	35.4 ± 6.5
Sesame	13.3 ± 4.7	35.1 ± 15.8
Marigold	15.2 ± 5.4	20.9 ± 4.3

Table 2. Qualitative analysis of allelochemicals in different intercrops using gas chromatography–mass spectrometry

Intercrops	Major allelochemical compounds
Bitter cumin	γ-Sitosterol, octatriacontyl pentafluoropropionate
Carom	γ-Terpinene, heptacosonal, quinic acid
Coriander	Phytol, quinic acid, 9,12,15-octadecatrienoic acid methyl ester, neophytadiene, 8-hexadecanal, 9-octadecanal
Fennel	D-Limonene, estragole, anethole, apiol, fenchyl acetate, phytol, 9,12,15-octadecatrienoic acid
Fenugreek	Methyl-mannose, neophytadiene, phytol, methyl stearate, ascorbic acid, 2,6-dihexadecanoate
Oats	Gramine, tryptophol, neophytadiene, phytol, hlucopyranoside, trytophan, γ-sitosterol
Pearl millet	Squalene, neophytadiene, dotriacontane, 9,12-octadecadienoic acid (*Z*,*Z*), methyl ester, oxirane, naphthalane
Sorghum	Pentadecanoic acid, 14-methylene methyl ester, 9,12,15-octadecatrienoic acid, dotriacontane
Desmodium	Maltol, benzufuran, methyl-mannose, neophytadiene, stigmasterol, γ-sitosterol, butyl 9,12-octadecadienoate
Sunnhemp	9,12,15-Octadecatrienoic acid methyl ester, phytol, dotriacontane, oxirane
Sesame	9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester, 9,12-octadecadienoic acid (*Z*,*Z*)-, methyl ester, hydroquinone
Marigold	Decanal, quinic acid

Table 3. Relative neighbour effect of different intercrops grown in *Bt*-cotton hybrid

Intercrops	2014	2015	2016	2017	2018	Mean over Farmers' Practice
Bitter cumin	–0.20	–0.20	–0.47	0.03	–0.28	–0.21
Carom	–0.04	0.03	–0.45	0.11	–0.22	–0.08
Coriander	0.22	–0.27	–0.44	–0.08	–0.43	–0.04
Fennel	0.06	–0.41	–0.49	–0.20	–0.29	–0.14
Fenugreek	0.09	–0.31	–0.47	–0.26	–0.53	–0.17
Oats	0.14	–0.27	–0.52	–0.18	–0.25	–0.09
Pearl millet	0.67	–0.18	–0.39	0.18	–0.23	0.33
Sorghum	0.30	–0.41	–0.49	0.05	–0.28	0.02
Desmodium	0.11	–0.15	–0.44	–0.02	–0.36	–0.06
Sunnhemp	0.56	0.42	–0.17	0.50	–0.12	0.42
Sesame	0.45	–0.20	–0.37	0.00	–0.25	0.17
Marigold	0.09	–0.31	–0.44	0.00	–0.44	–0.10

condition. Therefore, we assessed the RNE value of intercrops vis-à-vis the FP practice. Positive values were obtained with only four intercrops, namely sunnhemp, pearl millet sesame and sorghum, suggesting that these intercrops effectively suppressed weed emergence. This could be due to differences in the biomass as well as the allelochemicals released. Among the intercrops, sunnhemp, pearl millet, sorghum and sesame had greater plant biomass at the time of mulching. Although sorghum had greater biomass, it could not suppress the weeds effectively. This may be due to other factors such as greater soil moisture conserved. Further, although it is well known that the allelopathic compounds such as sorgoleone effectively controls weeds[27,28], it was not the case in this study. A probable reason could be that cotton–sorghum rotation is a common practice in the study region, and therefore, weeds may have become resistant to the allelopathic compounds. Also, pearl millet, sunnhemp or sesame are not grown here and thus the weeds may not have developed resistance to the allelopathic compounds released. Similarly, aromatic plants are not grown in the region, but did not show significant decline in the weed density compared to the FP treatment.

At the time of mulching, methanolic extracts of the intercrop samples analysed using GC–MS, showed the presence of several allelopathic compounds. The effective intercrops identified by RNE, namely sunnhemp, pearl millet and sesame, had some allelochemicals in common while some were unique. Medium- to long-chain fatty acids are identified allelochemicals[29,30]. Since linoleic acid and linolenic acid with their methyl esters were the key compounds present in sunnhemp, pearl millet and sesame, their abundance and release must have facilitated the inhibition of weed germination. Hydroquinone, a phytotoxic phenolic compound is another major allelochemical specific to sesame, which has been reported to inhibit seed germination and hypocotyl elongation in lettuce and leafy spurge when applied exogenously by disturbing the chlorophyll fluorescence and overall stomatal function[31].

Further studies are needed on the evaluation of individual allelochemicals and their weed control. For an allelochemical compound to be effective, its release should essentially correspond to the time of weed germination and emergence. It is probable that the release of chemical compounds may have been delayed due to stress or may have disintegrated into harmless products resulting in the weed seeds to germinate and emerge. This aspect needs further research.

Conclusion

In the present study, sunnhemp, pearl millet and sesame when used as intercrops with cotton, were effective in weed suppression. The combined effect of allelochemicals like fatty acids, fatty acid methyl esters, terpenoids and phenolics released from the intercrops proved toxic to the weed flora of cotton-based agrosystems. Thus, allelochemicals from intercrops can be exploited as eco-friendly herbicides for weed control in cotton-based cropping system. The reasons for loss of allelochemical potential of the compounds released from a few intercrops may be explored further.

Conflict of interest. The authors declare that they have no conflict of interest.

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