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Table: 1, Figs : 2

Allelopathy Field Research Methods. 1. Crops-weeds interactions

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ABSTRACT

Allelopathy techniques usually used under field conditions, may help in understanding and identifying allelopathic species and their harmful influences on other species are presented and discussed. New suggested technology enables the students and researchers to better understand the allelopathy mechanism in field are also included and explained. Suggested methods and presented techniques will help in separating the allelopathy from competition and allow better understanding of differences and similarities between both components of plants interference in nature. Advantages and weaknesses of each technique are mentioned, steps to overcome any problems in implementing each method are also provided and precautions are given where necessary. Alternatives to each technique that enable the researchers to confirm results obtained are also given.

Keywords: Allelopathy, field techniques, methodology, weed/crop interactions, suggested new technology.

1. INTRODUCTION

Allelopathy is only one component of plant interference in nature. It results from chemical interaction between plants through the environment and the allelochemicals are released from the plants in forms of volatiles, root exudates, foliage or root leachates and from decomposed plant materials in the soil. However, this mechanism is difficult to separate from other mechanisms of plant interference (e.g. competition). Therefore, conclusive judgment on allelopathic activity of certain plant species is difficult to justify in absence of a rigorous protocol allowing researchers to precisely designate or isolate this effect from others under natural conditions. Possible allelopathic activity of a plant species may be first observed from its distribution in the field and tendency of its individuals to aggregate together forming colonies in which plants of other species are almost or completely excluded (Fig. 1). In the case of allelopathic perennial woody weed species, absence of any vegetation underneath or in its vicinity is commonly observed. Individuals of these may or may not possess a strong interspecific competition. Sometimes there is no any other possible explanation except the release of phytotoxic chemicals to justify the normal growth of these individuals and the absence of individuals from other species. Individuals from a colony of an allelopathic species have a normal growth, while those of other

species closely growing to this colony suffer a great growth reduction. Growth reduction due to phytotoxins should be tested by higher supply of growth resources (water and nutrients) to the suffering plants, to evaluate their ability to recover from the negative effect and growth suffering they exhibit. Failure of individuals of the suffering species to recover and resume normal growth when more resources or growth factors are provided may be strongly explained by an allelopathic influence.

GENERAL OBJECTIVES

In these techniques and methodologies suggested for allelopathic studies under field conditions, I tried to introduce all interested people in this discipline of science and who are doubtful about the applicability of allelopathy under field conditions, to try and learn from the proposed techniques presented and to convince that allelopathy is an actual working mechanism in nature and can be studied and evaluated. In addition, other mechanisms of plant interference can be almost neutralized or excluded.

Aims of this paper are as under:

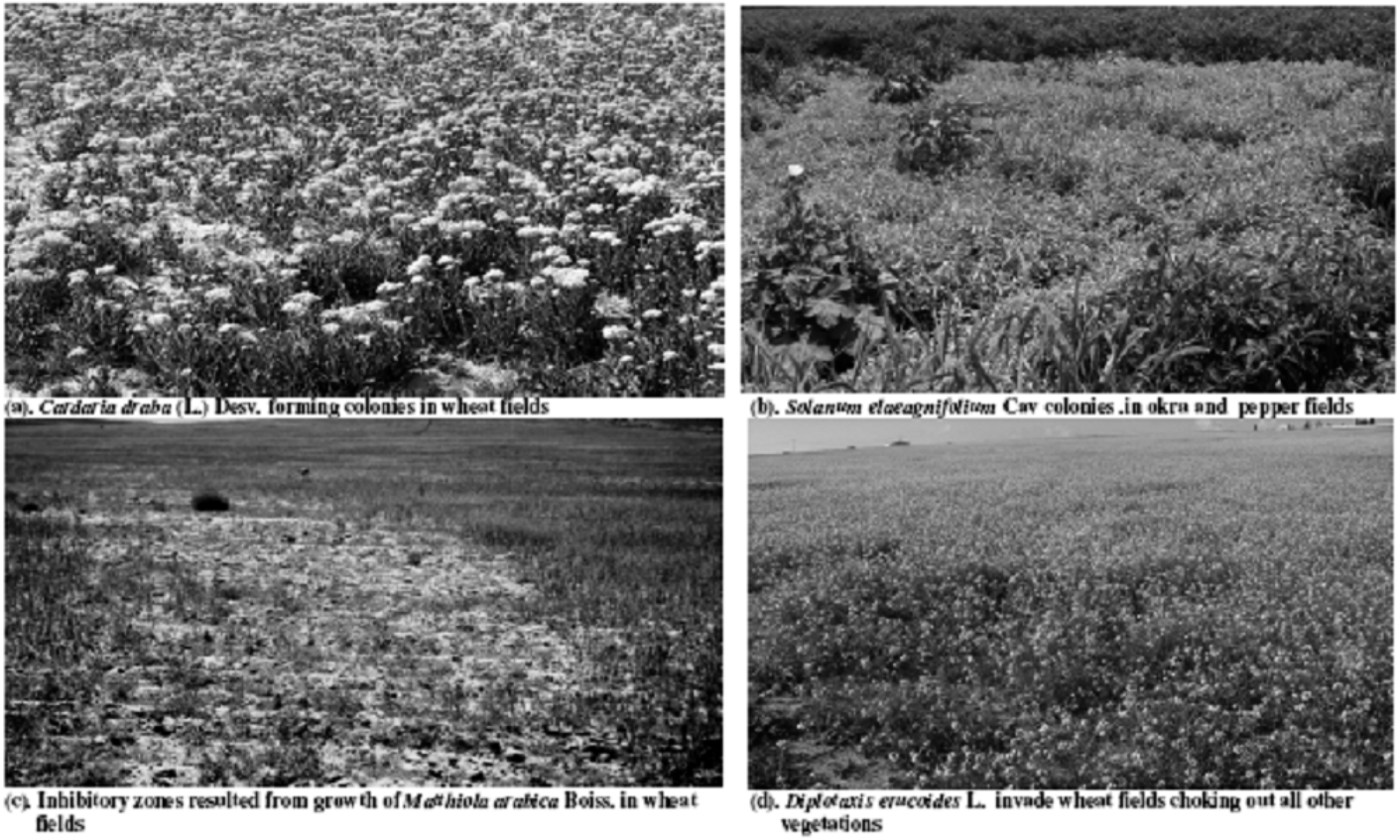
(i). Introducing some useful and helpful ideas to help readers themselves to diagnose the harmful effects of allelopathic species on others under field conditions.
(ii). Familiarize interested people with the proposed techniques and methods with tools and equipments required to separate allelopathy from other plant interference mechanisms.
(iii). Enable the readers' to fully understand allelopathy and how to implement each technique and isolate its harmful effects under natural conditions.

GENERAL PRECAUTIONS

In all mentioned and proposed techniques I tried to simulate as much as possible natural conditions, but none is perfect and all can be improved to better enhance the effectiveness in separating the allelopathy mechanism. Therefore precautions are required during the conduct of these experiments, while new ideas for improvement may come during the research and can be tested.

2. MATERIALS AND TOOLS REQUIRED

(i). **Laboratory Equipments and Apparatus:** Chopping machine, electrical oven, refrigerator, Waring blender, analytical balance, pH meter, EC meter, vacuum bump, centrifuge.
(ii). **Field Tools and Equipments:** A piece of land, a fruit tree orchard, sprayers, plastic sheets, plastic tunnels, small glasshouse units, plastic or glass cages, measuring tape, wooden stakes, soil tiller, hoes, harvester, fertilizers, source of water, cultivator.
(iii). **Miscellaneous:** Seeds of different crops and weed species, seedlings of different species, paper and plastic bags, aromatic essential oils, beakers, dark bottles, glassware, filter papers, digital camera, notebook, reference books, list of allelopathic species.



(a). *Cardaria draba* (L.) Desv. forming colonies in wheat fields

(b). *Solanum elaeagnifolium* Cav colonies .in okra and pepper fields

(c). Inhibitory zones resulted from growth of *Matthiola arabica* Boiss. in wheat fields

(d). *Diplotaxis erucoides* L. invade wheat fields choking out all other vegetations

Figure 1. Different allelopathic weed species showing inhibitory zones on wheat and some on vegetable crops

Table 1. Common and scientific names of plant species used

Common name	Scientific name
Alfalfa	*Trifolium* sp.
Barley	*Hordeum vulgare* L.
Black walnut	*Juglans nigra* L.
Branched broomrape	*Orobanche ramosa* L.
Calotrope or apple of sodom	*Calotropis procera* (Ait.) Ait.
Cucumber	*Cucumis sativus* L.
Faba bean	*Vicia faba* L.
Flax	*Linum usitatissimum* L.
Hairy vetch	*Vicia villosa* Roth
Inula	*Inula viscosa* (L.) Aiton)
Lemon grass	*Cymbopogon citratus* (DC) Stapf
Maize	*Zea mays* L.
Matthiola	*Matthiola arabica* Boiss.
Mint	*Mentha viridis* L.
Olive	*Olea europaea* L.
Onions (onion, garlic, leek)	*Allium* spp.
Pine	*Pinus* spp.
Plum	*Prunus* sp.
Potato	*Solanum tuberosum* L.
Purple sage	*Salvia leucophylla* E. Greene
Rice	*Oryza sativa* L.
River red gum	*Ecalyptus camaldulenisis* Dehnh.
Ryegrass	*Lolium perenne* L.
Silverleaf nightshade	*Solanum elaeagnifolium* Cav
Sorghum	*Sorghum bico*lor (L.) Moench
Tamarisk	*Tamarix pentandra* Pall.
Tiny Vetch	*Vicia hirsuta* (L.) Gray
Tomato	*Lycopersicon esculentum* Mill.
Western field dodder	*Cuscuta campestris* Yunck.
Wheat	*Triticum durum* L. or *aestivum* L.
White top	*Cardaria draba* (L.) Desv.
White wall rocket	*Diplotaxis erucoides* (L.) DC.
Wild oat	*Avena sterilis* L.
Witchweed	*Striga asiatica* or *hermonthica*

3. METHODS FOR STUDIES UNDER FIELD CONDITIONS

1. LIVE- INTACT DONOR PLANTS

1.1. Living mulch

This method is used to physically smother the weeds under field conditions. It is most widely used for fruit tree orchards, yet may also be applied in widely spaced field crops such as vegetables or field crops like maize. It is mainly implemented by growing a crop or other plant species between the rows of tree species to fill in the vacant space available for weed invasion. Species to be grown as living mulch should not compete with the main crop. Most used species are legumes such as *Vicia* spp. (*villosa* or *hirsuta*). These provide

nutrients and do not interfere with the main crop through competition. In addition other dwarf or tall growing species may be introduced between tree rows such as alfalfa, faba bean and onions etc. (Fig. 2).

(a) Broad been grown in *Prunus* sp. fields **(b) Alfalfa grown in *Olea europaea* L. fields**

Figure 2. Living mulch of two legume species in fruit trees.

Aims of this method are as under:

(i). Understand the effects of plant density on the effectiveness of allelopathic influences in the field

(ii). Understand the importance of density stress on donor plants and their ability to release higher amounts/ concentrations of allelochemicals to their surroundings.

(iii). Understand the density effects of receiver species in diluting the concentration of allelochemicals absorbed and dividing these chemicals in between its individuals.

Procedure

(i). Choose a fruit tree orchard. Divide the field into different sections each of 2-rows and use following treatments:

  (a). Keep one section (2-rows) untreated (control) in which weeds are allowed to grow in the vacant space between fruit tree plants.

  (b). In second section, weeds are controlled by continuous hand-weeding.

  (c) In rest of sections, grow legume crops (e.g. *Vicia hirsuta* or *villosa*) at different densities of your choice in the space available between tree rows.

(ii). After emergence of *Vicia* plants, keep observing weed growth between the rows of fruit trees. Record the names and densities of emerged weed species in all sections. Compare weed growth in different treatments and observe the performance of fruit trees (colour, development, growth and yield).

(iii). Compensate for the differences in nitrogen provided by different *Vicia* populations by adding nitrogen fertilizer to maintain the same N level for all plants in all sections. Add more N to the lower *Vicia* density and *Vice versa*. Notice that the denser the populations of living mulch, the better will be the growth and yield of fruit trees.

Absence of weeds from the space grown by *Vicia* plants and the severity effect on weeds with density is evidence for the smothering activity of *Vicia* on weeds, especially when accompanied with better growth of fruit trees, then we may conclude that this is likely due to allelochemicals released into the soil through root exudates.

(iv). Observe the growth of weeds in following season or year and compare with their growth in previously untreated control. If weeds are absent or their population is low compared to control or their growth is not satisfactory, this means that weeds are suffering from the residual effects of allelochemicals in the soil that left after *Vicia* was terminated. You can repeat the same treatments with different field crops or vegetables.

1.2. Volatile producing plants

The effects of certain volatile allelochemicals released by crop plants, able to inhibit the weeds growing between the crop species is another possible test. This phenomenon of inhibitory action can be easily observed on weeds growing in fields sown/planted with certain aromatic plants (emanating the essential oils into their surroundings) or Cucurbitaceae and Brassicaceae crops.

Aims of this method are as under:

(i). Test one of the common methods of allelochemicals release (volatile form) into the environment and the receipt of these volatiles by inflected species.

(ii) Introduce farmers to another possible method for weed management by growing crops which release allelochemicals in volatile forms.

Procedure

(i). Choose cucumber, squash or a *Brassica* species and grow in wide rows.

(ii). Compare the weed growth between the rows of these crops and growth of weeds between plants of other vegetable crops of similar size, planting distances and growth form.

(iii). Use different densities of allelopathic crops and observe the weed growth and crop growth and yield.

Precautions: Researcher should provide adequate resources for crops and treatments used

1.3. Crop accessions

Different crop accessions may also exert different effects on weeds. This situation however, may be due to allelopathy, competition or both effects. If dwarf and less vigorous accessions are associated with low number of weed species and density, the possible reason can be allelochemicals released by the accession plants.

Aims of this method are as under:

(i). Draw attention of allelopathy researchers to differences between the crop accessions in their ability to utilize growth resources available in the environment, which may be reflected as differences in their competition effects.

(ii). The differences between the accessions are due to competition or allelopathic effects or both.

(iii) Instruct the junior researchers on how to judge the effects observed in the field whether due to any component of plant interference or both.

Procedure

(i). Choose to work with rice, wheat or barley accessions.
(ii). Observe weed growth between different accessions.
(iii). Record existing weed species with each accession.
(iv). Take notes on growth and performance of crop accessions and associated weeds.

Precautions: (i). Sow all accessions at the same distance and densities.
(ii). All accessions should receive similar treatments and growth resources.

1.4. Plants density method

In this test, it is assumed that any sensitive allelopathic species may gradually recover from the inhibitory effects exerted on its growth from an allelopathic donor species by:

(i). Increasing the density of receiver species and thus diluting the concentration of allelochemicals taken up by its individuals. This gradually leads to less harmful effects of allelochemicals absorbed depending on the density used.
(ii). Reducing density of alelopathic donor species that leads to reduction in amounts and concentrations of released allelochemicals.

Aims of this method are as under:

(i). Test the effect of planting density of donor and receiver species in increasing or decreasing allelopathic effects on receiver individuals
(ii). To prove that allelopathy influences in the soil are allelochemicals concentrations-dependent.
(iii). Show possible ways to overcome the allelopathic harmful effects by manipulating the crop planting density.

Procedure

To verify the above hypothesis do following:

(i). Select a piece of land where an allelopathic species (e.g. *Sorghum bicolor*) was previously grown.
(ii). Choose a crop sensitive to the allelochemicals of the allelopathic crop e.g. maize.
(iii). Divide the land into different plots.
(iv). Use the following treatments in each of 3 replicates:
  (a) Maize grown at normal density,
  (b) Maize grown at different higher densities,
  (c) Maize grown in plots not previously cropped with sorghum.
(v). Add same doses of fertilizers to all plots.
(vi). Compare the growth of maize in all treatments.
(vii). Notice that growth of maize at normal density is not better than its growth at higher densities although resources are becoming more limited with higher densities (higher intraspecific competition).
(viii). It is important to notice that higher the maize density, the lower amount of allelochemicals may be absorbed by the individual maize plant.

(ix). Addition of nutrients at normal density was not enough to eliminate the effects of residual allelochemicals on maize plants.

2. DEAD-NONLIVING DONOR PLANTS (EXTRACTS/EXCRETIONS LEFT IN SOIL)

2.1. Dead mulch

2.1.1. Residues: Surface applied

Surface mulch may act through physical or chemical effects or both on weeds growth. Mulching of the soil surface with dead plant tissues may also conserve moisture, prevent direct sunlight and high soil surface temperature and thus may affect the microbial population and its activity.

Aims of this method are as under:

(i). Study the effect of plant residue left on soil surface on the growth and performance of crop plants

(ii). Learning to distinguish the effects of allelopathy from physical effects of plants residues left on soil surface.

Procedure

(i). Allelopathic effects of straw mulch may be tested by applying straw of wheat, barley, rice or ryegrass at certain thickness on soil surface planted by any crop species either vegetable or others from fruit trees. In all cases, untreated (control) plots must be always included to compare.

(ii). It is possible to examine the effects of straw mulch of different plant species at a single rate of application on a single species, or working with different levels of straw application of a single species. All agricultural practices employed should be kept uniform in all plots.

(iii). Observe weed growth in all treatments and compare weed species and density with untreated (control) plots. In another treatments, apply residues or straw of a non-allelopathic species and observe weed growth in all treatments.

(iv). Take notes on weed species, germination date and rate, growth in terms of height and vigorous and also on crop plants and yield under different treatments.

(v). If crop growth was improved and weeds density and/or growth was reduced, when straw mulch of low nutritional value was used, compared with crop and weed growth mulched with straw rich in mineral nutrients, then the cause is not physical or nutritional but rather an allelochemical. However, in this exercise the effects of straw mulch may be employed using fresh or dried residues.

2.1.2. Residues: Soil incorporated

Plant residues may release allelochemicals, nutrients or both and may also change the soil physical properties, improving water holding capacity, stimulate microbial populations and modify the soil structure. Therefore this technique was proposed to separate allelopathic effects of plant residue incorporated into the soil from any physical or mechanical effects of such residues on growth and performance of other species.

Procedure

(i). The allelopathic effects of residues from a plant species (e.g. wheat or ryegrass) on weed or crop growth could be tested by incorporating fresh or dry plant residues into the soil. This may be done using residue of a single plant species at different rates of application or by incorporating residues of different plant species and examine their effects on a single plant species.

(ii). Residues of allelopathic and non-allelopathic species could be tested on a target species used as an indicator. In all cases, control (residue-untreated) plots should be also included and agricultural practices should be kept at the optimum level of crop needs. Provide uniform supply of nutrients or water to crop plants in all treatments.

(iii). Take notes on crop plants height, vegetative growth or any signs of toxicity or negative effects on its appearance (e.g. yellowing, necrotic spots, burning and wilting). Note any differences in the effect of different residue rates used.

(iv). When residues of different allelopathic species were used, take notes on crop growth, compare crop growth in allelopathic and non-allelopathic plants residues and compare these with crop growth in residue-untreated control. Compare differences in weed species in plots received different doses or types of plant residues.

(v). Take notes on weed species present, their densities, growth and performance. Note that absence or lower weed growth and performance in plots with high amounts of plant residues means higher allelopathic effect. Poor weed growth and normal or better growth and performance of crop growth when less nutritive plant residue were used and soil-incorporated, means that the effect is most likely due to allelopathy.

Precautions: Dried residues may reduce the water holding capacity of soil, soil texture may becomes too hard for roots to penetrate and thus results in differences in the germination and /or growth of indicator plants, which are due to physical effects rather than allelochemicals. Be sure that moisture is normal and adequate in soil- incorporated residues.

2.2. Crop rotation

Crop rotation has its distinctive effects on weed population associated with crop plants. The first effect is the breakdown of weed population with time. Every time different crops were introduced into rotation, more reduction was observed in weed population density and growth since specialized weeds tend to increase in density and growth in absence of crop rotation system because certain weed species usually conjugate with certain crops of similar morphology, physiology, growth requirements and of genetic relations.

Aims of this method are as under:

To separate the allelopathic effects of root exudates left in the soil and from the residual effects of depleted resources by previous crops in rotation.

Procedure

(i). The effect of allelopathic species on crops or weeds in rotation could be directly examined under field conditions by including an allelopathic species in a rotation system and observing the germination or growth of subsequent crop species in rotation

compared with its growth alone or after other non-allelopathic crop species in rotation. It is also possible comparing the weed species and growth after each species in rotation.

(ii). The growth promotion of receiver species in rotation may be due to the release of growth promoting substances (nutrients and hormones) from the donor species grown before or may be due to the negative effects on weed germination, growth and population.

(iii). If growth of following crop species in rotation was reduced and some negative signs were observed on its appearance, then the effect may or may not be due to allelopathy. This situation however, may be further verified by supplying higher level of nutrients and water to the effected plants and observing crop plants recovery.

(iv). If plants were recovered from the symptoms exhibited, then allelochemicals may interfere with nutrients or water uptake from the soil or in their translocation through the plant system. When plants failed to respond to the higher supply of growth resources then the effect may be concluded as a strong allelopathic influence on inflicted species and is not reversible or possible to overcome by applying more growth resources since affected the physiological functions and/ or biochemical process inside the plant itself.

Precautions: There should be no resource limitations after growing of certain species in rotation and to ensure that the observed effects in the field are not due to less supply of any growth factor.

2. 3. Root exudates

Root exudates release hundreds of chemicals into the soil and influence the plant growth. Some of these chemicals may promote or inhibit crop growth; others are nutrients that could enhance crop growth or microbial populations. Root exudates may have different pH value, chemical nature and thus their effects may vary on the surrounding environment, seed germination and weed growth. However, these chemicals are different in their activities, persistence, half life in the soil and quantities released into the soil. They could enhance or inhibit the uptake of nutrients and water from the soil solution and may interfere in seed germination and seedling growth. The effects of root exudates may be in part similar to crop rotation, since in both cases root exudates are released into the soil and thus are involved in the effect on inflicted species.

Procedure

(i). Allelopathic plants can be grown in field plots. After removing these plants at a specific growth stage, soil is prepared to grow a receiver plant species.

(ii). Plots of indicator plant species must be included and cultivated in plots not previously cultivated by the allelopathic species and included as a control. Comparison in germination, emergence and growth of crop plants in different plots should be carried out. Observe growth of sensitive (receiver) species after different allelopathic and non-allelopathic species and/ or its growth after a donor allelopathic species planted for different periods of growth or growth stages before was removed.

(iii). Any differences in growth of receiver species may be due to the release of promoting (positive effect by release of nutrients or growth promoting substances) or inhibitory

(negative effects through inhibitory chemicals) substances. The negative effect may be due to the exhaustion of nutrients or moisture from the soil by the formerly grown species prior to the sowing of sensitive crop (receiver). This however, may be easily overcome by higher supply of water and nutrients to compensate for the resources removed by the previously grown donor species.

(iv). Certain crops never grow or yield normally when follow certain crop species (e.g. maize and sorghum) in rotation. It is always observed that maize yield is the lowest after sorghum than after other crop species or separately in previously uncultivated soil. Failure of resource supplies to overcome this effect may be explained by allelochemicals produced and released through root exudates into the soil by sorghum plants grown prior to maize cultivation.

(v). The best way to examine the effect of root exudates may be through testing their effects on germination, attachment and emergence of a parasitic-germination (stimulant-requiring) weed species such as *Orobanche* or *Striga*. A trap species such as flax (or sorghum for *Striga*) may be chosen to grow in a heavily infested soil by *Orobanche ramosa* parasitic weed. Plots uncultivated by flax are included as a control. At seeding stage of flax, plants must be harvested from the above soil level, the soil then is tilled (loosened) and the seedbed is prepared for tomato planting. Seedlings of tomato are transplanted as done by local farmers. Plots uncultivated previously by flax plants are also prepared and transplanted by similar number of tomato seedlings using the same planting distances (control). During the growing cycle of tomato keep observing tomato growth, flowering and fruiting time, *Orobanche* emergence time and intensity of infestation in each plot and in all treatments. At harvest, tomato plants are removed by cutting the above ground vegetative parts and excavated the below ground root system. Wash carefully with running water after placing the harvested tomato plants in a fine mesh sieve and observe *Orobanche* growth and infestation on tomato roots in different treatments. Harvest, and then count the *Orobanche* plants and haustoria on each tomato plant root system in each plot. Harvest emerged *Orobanche* shoots above the soil level and compare their growth and number with those harvested from tomato in soil-infested but not previously grown by flax (control). Calculate number of *Orobanche* on roots (not emerged), above soil level (emerged) and their dry weights, total number (above and below soil level), tomato height, and dry weights of roots and shoots in all treatments. Tabulate and statistically analyze the data for differences between treatments and explain the results obtained in terms of the effect of root exudates of flax plants on *Orobanche* infestation to the following grown tomato plants.

2.4. Soil extracts

This method aims to study the effects of soil extracts containing the allelochemicals on growth of receiver species.

Aims of this method are as under:

(i). Show the researchers, that allelochemical released into the soil water or soil particles can be detected,

(ii). Isolate and prove the phytotoxic effects of soil extract or isolated allelochemicals on growth of sensitive species.

Procedure

Select a well known allelopathic species that leach allelochemicals from its foliage parts or through root exudates into the soil. Such species may be easily identified by observing the vegetation underneath their foliage parts. Some allelopathic species included pine, walnut and eucalyptus. Of perennial weeds, you may choose white top (*Cardaria draba*) and *Salvia leucophylla*. However, since shading may also affect the growth of underneath grown species, while some species are shade tolerant, it may be worth sowing seeds of different plant species or weeds at same seeding rates, underneath allelopathic and non-alleloapathic species. Control plant species must be selected as offering similar or higher shading effects than allelopthic species. Record emerged plant species in both cases and their density and names. Keep observing growth and development of emerged species underneath allelopathic and non-allelopathic species; record any changes in the appearance and growth of species in both places and cases. Any negative or abnormal growth should be explained by the effect of allelopathic chemicals deposited into the soil.

Procedure

(i) Collect soil samples from under both plant species (allelopathic and non-allelopathic), extract the soil in the laboratory by soaking a known amount of soil from both treatments in a known volume of water.
(ii) Shake soaked soils for certain time. Filtrate soil solution through filter paper on Boucher funnels, collect soil solutions, measure the pH and EC of both solutions, and irrigate two plots sown by inflected species with water extracted from soil obtained from underneath allelopathic species and the other two plots by water extracted from soil obtained from under non-allelopathic species.
(iii) Compare the germination of treated species in both treatments, observe seedlings emergence and record their time of emergence, Record any differences or symptoms in emergence or growth and development of different sown species and compare vegetation density, growth and development in both treatments.
(iv) Provide a liquid fertilizer to all plots and keep watching if the effect observed on inflected weeds irrigated with soil-solution obtained from under allelopathic species was overcome and plants were recovered from the symptoms exhibited on their foliage parts.

2.5. Plants extracts

Aqueous or alcoholic extracts from plant parts may be used to control weeds by direct application on their foliage parts or by adding these extracts into the soil. Phytotoxicity of plant extracts depends on species extracted, concentration used and time of application with regard to treated weed growth stages. It is well established that young weed seedlings are most sensitive to herbicides and better respond to these chemicals than mature old weeds.

Procedure

(i) Choose a plant species from literature previously depicted as allelopathic on weed species (Qasem & Foy, 2001). Collect their healthy vegetative parts and record their growth stages at collection time.

(ii) Wash the aerial parts with water, then with distilled water and chop the plant material into small segments using a pruning scissor. Add 300g of chopped plant material of each species into a liter of distilled water in a Waring blender bottle. Blend the mixture for 5-10 min until a homogenized solution is obtained.

(iii) Allow the whole mixture to stand for 30 min, and then filtrate the supernatant of the plant material through a Buchner funnel lined by a Whatman no. 1 filter paper. The filtrate may be regarded as a full strength extract solution. Different amounts of plant materials per liter of distilled water could be used to concentrate or dilute as required. It depends on the strength of phytotoxic effects of the allelopathic plants.

(iv) Determine the pH and EC of extracts used, select a piece of land usually infested by different weed species, or artificially infest the soils by seeds of different known weed species using the same sowing rates in all.

(v) After emergence divide the land into different plots, using 3 plots per treatment (replicates). Treatments should be randomly distributed in the field, for each treatment (3 plots) assign an extract of one allelopathic plant species. Consider one treatment as untreated with extract but instead with water (control).

(vi) Treat small weed seedlings in all plots with extracts of the allelopathic plant species. Observe extract effects on the appearance and growth of weed species in each plot and compare their growth with that of untreated control. Differentiate the responses of different weed species within and among treatments and record the differences. At mature stage or at certain time after treatments, record any biological measurements required and harvest all plants per plot, oven-dry weeds vegetative parts at 70^{o}C for 48h and determine their dry weights. Tabulate and statistically analyze the data and prepare a report on the effects of extracts used on different weed species. It is possible using sorgaab (source: *Sorghum bicolor*) aqueous extract against wild oat (*Avena sterilis*) infesting wheat fields or aqueous or organic solvent extracts of *Inula viscosa* as direct spray on the parasitic dodder (*Cuscuta campestris*) plants, or extract of *Calotropis procera* directly sprayed on small weed seedlings underneath fruit trees.

Precautions: Plant extracts used in mixture with herbicides to reduce the rate of herbicide and the partial substitute of herbicide by plant extracts, may act by improving the absorption of herbicide molecules and thus work as surfactants rather than toxic materials themselves.

3. LIVING DONOR AND RECEIVER PLANTS MIXTURE

3.1. Intercropping systems/Crop mixtures

This system is usually implemented by growing different crops in mixture in the same field for the purpose of increasing land productivity and weed control. Usually crops of different growth forms, habits and requirements are selected to grow together and each may supplement the other in its requirements. Therefore, each species complement the needs of the other for environmental resources and thus a better use of growth resources. Crops growing in this system include monocots and dicots, legumes and cereals, and short and high statured crops. The greater the differences between crop species requirements and their differential responses to different growth factors the better the results and yield obtained. Perhaps this may be due to competition or no competition possibility/situation

between species for growth factors or differences in their growth from the above and below soil growing systems and the extent of the differences between intercropped species. However, these differences are usually reflected on yield and yield components. In all cases, if yield in intercropped plots is higher than sole crop, it means that the interaction is positive at which one of the species in mixture may provides promoting substances to the other species or both have differential requirements and thus not in direct competition over resources. When the yield in mixture is lower than that of sole crops separately, a negative interaction between intercropped species occurs which may be due to competition effect or allelochemicals released or both. Failure to enhance growth and yield of this mixture above that of a sole species by higher supply of growth factors, means that other factor/s are responsible on the low growth and yield obtained which is most likely due to allelochemicals release into the soil. The effect may be also due to emanating volatile chemicals into the surrounding environment which may affect or stunt growth of the accompanied species in mixture. However, the effect may be also extended to reduce growth and population of sensitive weeds found in this system.

3.2. Plants volatiles

This technique depends mainly on the use of plant species that release volatile allelochemicals to the surrounding environment. Aromatic plants of Labiataeae and Umbelliferarea families and others such as Cucurbitaceae produce volatile allelochemicals

Procedure

3.2.1. Plant volatiles in the field

(i). A cucumber accession showing allelopathic activity against weeds might be used and compared with non-allelopathic lines or cultivars in their effects on associated weed species. Volatile chemicals may be directly absorbed by aerial parts of sensitive species and thus interfere with their growth and development. They may also reach the soil, dissolved in irrigation water from the above ground level or in dew droplets and affect germination of other species.

(ii). The allelopathic influence of these species could be observed as bare zones surrounding the donor plants (e.g. *Salvia leucophylla*) or as absence of plants underneath the top of donor plants (e. g. *Ecalyptus camaldulenisis* or *Pinus* spp.).

3.2.2. Volatiles from essential oils in the field

(i). In the open field, choose a piece of land heavily infested with weeds. Treat half of this land with the emerged small weed seedlings by spraying essential oil of lemon grass (*Cymbopogon citratus*) and keep the other half untreated.

(ii). Observe any changes or symptoms on the foliage parts of the sprayed weed species, record these changes. Explain any phytotoxicity observed on treated weeds and follow up any subsequent changes in weed populations.

3.2.3. Volatiles using small glasshouses or cages

(i) This experiment may be performed inside a small glasshouse of 2 x 2 m, where an aromatic plant is sown together with seeds of weed species. After emergence of crop and weed seedlings, some units of glasshouses are opened and others remain closed as long as possible during the growing period.

(ii) Essential oils of the aromatic plants may be also frequently sprayed into the air inside the small glasshouses sown with seeds of weed species. Seeds sown in other set inside of small glasshouses remain untreated. In each treatment, count the number of weed species emerged and their germination percentage and observe the growth of emerged weed seedlings for all species in vapour treated and untreated glasshouses.

The same test may be conducted using glass or plastic small cages placed in the glasshouse or directly in the field at which plants in certain cages may be treated with aromatic oil while others are not (untreated control).

3.3. Plant foliage leachates

Allelopathic effects due to foliage leachates is easily detected by observing germination or growth of receptor plant species inside patches of donor species or the growth of receptor species in the vicinity of the allelopathic species. Some allelopathic plants able to release foliage leachates are *Juglans nigra* L., *Mentha viridis* L., *Cardaria draba* Desv. and *Tamarix pentandra* Pall. However, leachates may be phytotoxic or not upon the release from foliage parts. Some become only toxic when reach the soil and interact with microorganism. Others may be toxic once released and before reaching the soil. However, it is worth mentioning that leached materials are liable to be changed from toxic to nontoxic and *Vice versa*.

Procedure

3.3.1. Mint plots

(i) Choose mint (*Mentha viridis*) planted plots in the field and split these into two groups. In the first, irrigate mint plants by a hand-held hose or a spryer from the above foliage parts (try to wash the vegetative parts of mint plants at each time of irrigation). In the second group of plots, irrigate mint plants using surface irrigation system (water running above soil surface) observe growth and performance of mint plants in both groups.

(ii) Keep watching the stand of mint in both treatments. Mint plants irrigated from the above foliage parts may start declining in growth and disappeared after certain period. This decline or plants absence in these plots is only explained by the effect of foliage leachates on the individuals of this species (autopathy). Replace certain number of mint plants in each plot by sowing similar seed number of wheat per plot (heteropathy). Wait for a while until wheat emergence. Take your notes on wheat germination percentage and time of emergence.

(iii) When seedlings reach 10 cm height. irrigate all plots in the same way you started with when only mint plants were grown in plots. Observe growth and development of wheat plants in all plots, compare wheat growth and stand in plots irrigated through sprinkler or foliage parts and those on surface-irrigated soil. Explain any differences in growth and performance of wheat plants in both by allelopathic influence of mint plants through their foliage leachates leached to soil surface.

3.3.2. Wheat plots

(i) In another test, observe growth of wheat plants within and around the colonies formed by *Cardaria draba* in wheat fields. This was explained by allelopathic effects of foliage leachates on wheat plants (Qasem, 1994).

(ii) Choose and examine the different allelopathic species reported to express this effect through the chemicals released from their foliage parts as leachates in irrigation water or rainfall.

3.3.3. Potato planted under walnut trees

In this case, observe the potato plants growth underneath walnut or apple trees. Take your own observations on growth and performance of these potato plants.

Precautions: Always provide same amount of water in each irrigation and to all treated plots. Note that other materials or chemicals may be also leached with foliage leachates such as plant hormones, minerals, ketons, proteins, aldehydes and other organic molecules etc.

CONCLUSIONS

One of the challenges faced by allelopathy researchers is the lack of a suitable experimental protocol that enables separation of allelopathy from competition in nature. A large number of techniques have been developed and many others were suggested that help interpretation of the results obtained at least in part as due to allelopathy. On the other hand, a large number of phytotoxins were isolated, characterized and biologically tested against different agricultural pests or species. In spite of the progress achieved in allelopathy research, isolation of this mechanism of plant interference under field conditions from other plant-plant interactions remained to be solved. Unfortunately, several investigations claiming allelopathy in the field failed to explain the observed evidences as solely due to allelopathy. An effective experimental design allowing separation of allelopathy from competition remains a far reaching task. Most of the procedures presented in this work imply long term investigations to show allelopathy and cannot be conclusive of occurrence of allelopathy in the field. Nevertheless, they help to diagnose the effects exerted by this mechanism. All presented procedures are liable to be improved, revised or totally changed and any suggestions aim to improve isolation and diagnosis of allelopathy mechanism are extremely important and most welcome.

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SUGGESTED READINGS

1. Blair, A. (2004). Plant-plant allelopathic interactions: Do they occur? 13 pp. Available at :http://www.colostate.edu/Depts/Entomology/courses/en570/papers_2004/blair.pdf Accessed on 16 December 2009.
2. Blum, U. (2007). Can data derived from field and laboratory bioassays establish the existence of allelopathic interactions in Nature? In: *Allelopathy: New Concepts and Methodology*, (Eds., Y. Fujii and S. Hiradate), pp.31-38. Science Publishers, NH, USA.

3. Blum, U. (2011). *Plant-Plant Allelopathic Interactions*. Springer, Berlin. 200 pp.
4. Carral Vilarifio, E.V. (2002). Trends in allelopathy research over six-year period analysis (1995-2000). *Allelopathy, From Molecules to Ecosystems*, (Eds. M. J. Reigosa and P. Nuria), pp. 299-304. Science Publisher, Inc. Enfield, USA.
5. Harper, J.L. (1977). *Population Biology of Plants*. Academic Press, London.
6. Mallik, A. (2005). Allelopathy: Advances, challenge and opportunities. In: *Proceedings, IV Congress on Allelopathy*, (Eds. J.D.I. Harper, M. An, H. Wu and J.H. Kent), pp. 3-11. Charls Struct University, Wagga Wagga, NSW, Australia, 21-26 August, 2005, International Allelopathy Society.
7. Narwal, S.S. (1994). *Allelopathy in Crop Production*. Scientific Publisher, Jodhpur, India.
8. NI, H. and Zhang, C. (2005). Use of allelopathy for weed management in China- a review. *Allelopathy Journal* **15:** 3-12.
9. Pratley, J.E., An, M. and Haig, T. (1996). Following a specific protocol establish allelopathy conclusively - an Australian case study. *Allelopathy, Science for the Future*. Vol.1. (Eds. F.A. Macias, J.C.G. Galindo, J.M.G. Molinillo and H.G. Gutler), pp. 63-70. Servicio De Publicaciones Universidad De Cadiz, Cadiz, Spain,
10. Qasem, J. R. (1994). Allelopathic effects of white top (*Lepidium draba*) on wheat and barley. *Allelopathy Journal,* **1(1):** 29-40.
11. Qasem, J.R. (2010). Allelopathy importance, field application and potential role in pest management: A review. *Journal of Agricultural Sciences and Technology*, USA **4**: 104-120.
12. Qasem, J.R. (2010). Differences in the allelopathy results from field observation to laboratory and glasshouse experiments. *Allelopathy Journal* **26:** 45-58.
13. Qasem, J.R. and Foy, C.L. (2001). Weed allelopathy, its ecological impact and future prospects. *Allelopathy in Agroecosystem*, (Eds. R.K Kohli and H.P. Singh), pp. 43 -119. Haworth Press, USA,
14. Qasem, J.R., and Hill, T.A. (1989). On difficulties with allelopathy methodology. *Weed Research* **29:** 345-347.
15. Reigosa, M.J., Pedrol, N., Sanchez-Moreiras, A.M., and Gonzalez, L. (2002). Stress and allelopathy. *Allelopathy, From Molecules to Ecosystem*s, (Eds. M.J. Reigosa and N. Pedrol), pp. 231-256. Science Publisher, Inc. Enfield, USA,
16. Rice, E.L. (1974). *Allelopathy*. Academic Press. New York.
17. Rice, E.L. (1984). *Allelopathy*. 2nd Edition. Academic Press, INC. London.