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Research Article

A New Technology Separating Allelopathy From Competition in Pot Experiments

Abstract

A new technology is developed to separate allelopathy from competition in pot experiments. Square pots of any sizes may be used. Each pot is divided by a metal or pvc mesh (100-150 micrometer) barrier fixed from the bottom to pot surface. The barrier divides the pot into two sections filled by the same amount of soil mixture. Seeds or seedlings of the donor and receiver species can be planted separately each in a pot section. Density of the donor plant can be varied as required. Nutrient solution can be equally and uniformly added to both pot sections. At an extended period, another similar barrier can be also inserted from the above pot surface to prevent shoot competition. In the control, both receiver and donor plants can be separately grown in divided pots and treated similarly as in other treatments. The technique prevents donor invasion into receiver section and differentiate non-allelopathic from allelopathic species. Failure of receiver species to perform or attain normal growth may be regarded as an indicator on possible allelopathy effects of the donor species. All kinds of comparisons and combinations can be tested and under both normal and stress conditions. A diagram of the new technique separating allelopathy from competition is illustrated.

Introduction

Allelopathy is not accepted among ecologists and many have argued that its effects cannot be separated from other mechanisms of plant interference mainly competition. Allelopathy is a direct negative chemical effect on one plant resulting from the release of allelochemical into the environment by another while competition is a struggle between both on one or more growth factors in limited supply [1]. A great effort was spent to differentiate between competition and allelopathy effects by many researchers during 1970s, while in the 1990s others appeared convinced in that the effects were often interdependent and could not readily be separated [1,2]. In spite of all these difficulties, a large number of publications on different aspects of allelopathy are accumulating yearly. Different methodologies or techniques are commonly used while researchers keep theoretically struggling to isolate allelopathy from competition and thus to proof allelopathy role and existence in the real life. Regrettably, methods followed in a large number of publications were exactly the same used at early stage of this science and simulated the same historical phenomona with lack of actual and strong scientific evidence on their natural existence or field application. Most were based on the use of plant extracts in Petri-dishes under laboratory conditions; less number in glasshouse potted- soil and the least were reported from field studies [3]. Many articles were concluded by similar implications on allelopathy effect as were early reported. This violate researchers, worldwide, deep concern and criticism on results claimed as due to allelopathy. Most results were theoretical, descriptive and based on observed symptoms and the morphological severity of allelopathic effects with no supporting firm evidence from nature's allelochemicals [4] but a rather reflection of deep realization and speculation. Some were entirely reported cases from natural or agroecosystem (most based on visual observations) on the suffering of certain plant species from another and explained as due to allelopathy. However, one of the misleading conclusions is that based on results derived from laboratory studies as in general were mainly conducted using plant extracts, root exudates or foliage leachates of dead plant tissues on artificial media. Extracts are commonly used in allelopathy studies to explain the observed harmful effects of certain plant species on other/s under field conditions [1], and different pioneer researchers are not convinced by the results obtained using this technique [5,6]. Although it is simple, widely used and preferable but its credibility is highly questionable since extracting all cell materials including releasable and unreleasable chemicals under normal conditions [7,8]. Techniques followed are widely dependent on researcher's objectives, target plants, conditions, and facilities. Screening methods conducted in laboratory using substituting artificial cultural media such as agar, sand or hydroponic culture, not applicable to natural conditions, would lead to misleading results. For instance, the common

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disadvantage of plant box method [9], relay seeding technique [10] and equal compartment agar method [11] is the use of agar as the culture medium, which assumed that allelochemicals would directly act on the target plants, thus overlook any possible modification of these chemicals in soil. Fujii et al. [12], developed "rhizosphere soil method" for the evaluation of allelopathic activity of plants, which is agar medium included dry leaves of donor plants or culture soil and seeds of the target plant are placed on the surface of agar medium opposing the spirit of allelopathy science.

Rigorous proof of such experimental results under glasshouse and field conditions is necessary to avoid any misleading results and false conclusion on the allelopathic activity of certain plant species. Willis [13], listed conditions required to provide evidence on alleloapthy operation and even though all do not prove that allelopathy is operative but only offers the most reasonable explanation of the observed pattern.

Although workers claimed "based on techniques used" or assumed that results obtained are due to allelopathy under specific conditions but critical analysis and deep thinking in the used techniques may reveal more involvement of competition [5,14]. Since separation or complete isolation of competition from allelopathic effect is difficult in the field but a well and carefully designed technique remained possible to develop considering positive and negative aspects involved in plants interactions in nature. However, as mentioned earlier the available techniques for allelopathy studies are many and varied [5,15,16] and some have been already reviewed, evaluated and previously criticized [5,15]. While most, if not all, followed in laboratory studies are artificial and do not represent actual natural situation, others failed to eliminate allelopathy interference/overlapping with competition or lack natural conditions or requirements to operate. In addition, different factors may be also involved in the effect obtained using such techniques. For example, with use of glasshouse potted soils or hydroponics designs more problems emerged which may negate allelopathy existence and thus failure to eliminate competition interferences. For example, pot screening method [17] without the use of pre-germinated seeds may result in varied densities of both donor and receiver species, whereas allelopathic activity is density dependant [18,19]. Considering all these difficulties, a drip irrigation technique was developed and introduced to separate competition from allelopathy between weeds and tomato plants grown in pots placed in channels and irrigated by nutrient solution [20]. These workers demonstrated that one weed species has an allelopathic effect through root exudates released into the circulated nutrient solution while similar effect of the other weed species was not possible although both were reported as of allelopathic influence.

Field studies also failed to separate allelopathy from competition using smother or highly competitive crops in forms of living or dead mulches or their cropping systems in intercropping design and more complications were added and challenge separation of allelopathy from competitions. A recent thorough review of applied allelopathy for weed management is available (14], allelopathy techniques were also reviewed and evaluated [15] and new proposed methodologies and techniques were suggested. Moreover, differences in results obtained between laboratory and glasshouse experiments and between these and some observed natural phenomenon on certain weed species were reported [15].

In the present work, a new technology that allows better separation of allelopathy from competition between different plants species in potted-soil under glasshouse condition is introduced, sketched illustrated and discussed.

Materials and Methods

Description of the technique

PVC square pots of 20 by 20 cm may be used in this technique with holes made for drainage in the bottom. A compartment (barrier) of the same pot height, made from metal or net mesh of 50-150 micro-millimeter pores and hard enough to withstand soil pressure can be inserted from pot surface down to the bottom. This compartment divides the pot into two identical sections. The net/perforated compartment may be also made from different materials (leather, jute, PVC or plastic}. If made from PVC, then it can be built in with the pot or slides inside from the bottom to increase or decrease space allocated to donor or receiver plants. The pore size can be varied according to roots diameter or thickness of studied species with the purpose of prevention root physical intermingle or penetration into the section of the partner species. This would keep roots of receiver plants separated from those of the donor and the only contact between both is mainly through root exudates possibly containing allelochemicals from donor plants, partially leached along the opposite pot section and received by the receiver plants through the mesh compartment. The pot may be vertically placed or made more or less sloppy toward the receiver section to allow normal flow of exudates into the pot section grown by the receiver species. Flaw of exudates may occur along the whole perforated barrier. This technique also allows planting donor and receiver plants at different levels (heights) in the pot and comparison of receiver plants performance when donor plants planted at different heights or in different section sizes.

In order to avoid any limitations in mineral nutrient supply, a full strength nutrient solution can be added weakly to each pot section and plant responses may be kept under observation throughout the whole study period. Moisture level in the soil can be maintained by adding water for each section, separately. The same amount of water is also applied to all pots and sections or may be made differ between receiver and donor plants based on their requirements. The technique allows studying different densities of donor species and examining their influence on germination and growth of the receiver plants. It may be also used to study the effects of root exudates of donor plants introduced into the system as transplants and examine their effects against receiver species grown from seeds or seedlings.

Since stress conditions are important in production of alleloachemicals [21], nutrient solution may be added at different strengths or suspended only on donor plants to

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eliminate any possible nutrient shortage and intraspecific competition between individuals of this species. Water supply may be also varied to donor plants but not to the receiver. Densities of both receiver and donor species can be made vary as needed to increase or decrease allelochemicals concentrations. This would concentrate allelochemicals released from donor plants and received into receiver section or *vice versa*. Differing receiver density may also work well with density dependent allelopathy technique [22] and thus can dilute or concentrate the amount of allelochemicals absorbed by receiver individuals.

Soil mixture in pots may be also varied at which sand, clay, peat ratios are manipulated. Charcoal can be added to absorb some of the released allelochemicals into the soil and at various percentages. Roots of receiver plants could be harvested from relevant pot section and their features and morphology are then examined.

Where plants grown for a long period and shoot interference is possible, a higher barrier mesh/perforated compartment can be used that divides the pot from below and above soil surface and thus separate shoot and root competition between donor and receiver species.

Section size grown by donor and receiver species may be increased or decreased as required to eliminate any effect of space or spatial arrangements of both donor and receiver individuals or minimize its importance.

Control of receiver plants is transplanted or sown using the same divided pots filled by the same amount of soil but only in one section (half) of the pot and plants similarly treated in absence of donor plants, or donor species is replaced by a more well known competitive species in order to confirm that no involvement of competition on the effect obtained on receiver individuals grown by allelopathic donor.

Results

The diagram of the technique is drawn and illustrated in Figure: 1

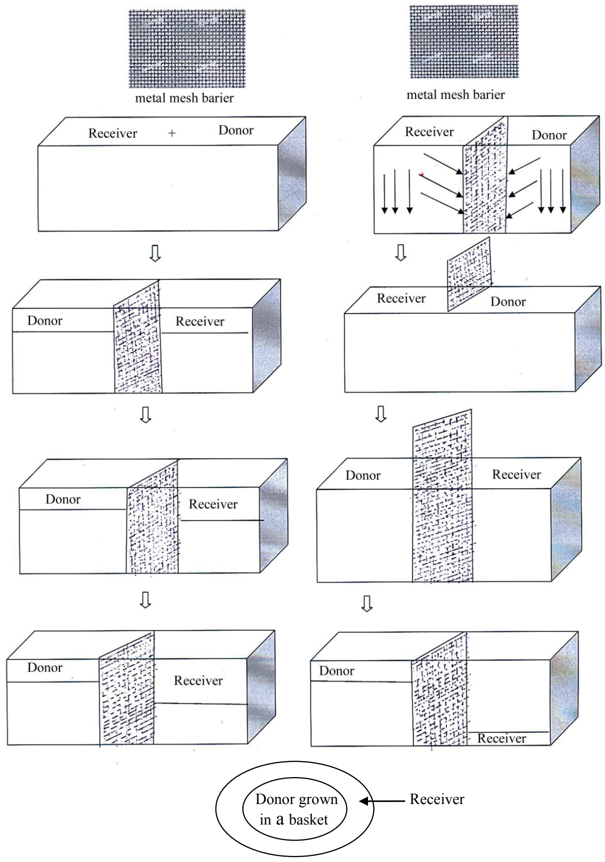

Figure 1: A schematic diagram illustrating the metal mesh barrier and divisions of square-shape pots allowed root separations, shoot separation, root and shoots separations (full separation), no separation, donor and receiver species planted at different depths relative to each other and donor planted in basket-shaped mesh barrier inserted into a larger pot grown by receiver species.

Advantages of the technique and possible development

These can be summarized as follows:

Root competition is prevented or minimized and thus no possible depletion of resources occurs by donor plants could affect the receiver root zone. Therefore root zone overlapping is not possible.

Growing donor and receiver plants separately in two sections of the pot prevent physical movement and intermingling of roots and possible smothering effect of one on another, while soil in each section can't be exploited by the roots in the opposite section. The barrier layer can be doubled or tripled according to root diameter or thickness.

Shoot competition is prevented by insertion of above ground compartment between donor and receiver species.

Allelopathy influence can be intensified or reduced as needed by increasing or decreasing donor density or manipulation the supply of growth factors.

Root growth and density of donor and receiver species is increased or decreased by controlling the space allocated to each species per pot. The pot section given for root growth of any can be increased or decreased as needed by changing the place of the barrier inside the pot or sliding the barrier into the appropriate position. Thus root volume may be increased or decreased according to restriction extent of the root system of grown species.

Density dependent allelopathy can be also tested by increasing or decreasing density of receiver species and study the performance of its individuals while concentration of allelochemicals remained the same by fixing donor density.

The technique allows testing the effect of allelopathy at different growth stages of receiver or donor plants.

Stress conditions can be created as required to increase allelochemicals production and secretions and thus the role of environmental factors.

Supply of nutrient solution eliminates any possible inter- and intra-specific competition in donor or receiver individuals.

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Charcoal absorbs allelochemicals and can be added to the soil mixture at different percentages and study performance of receiver plants accordingly.

Pots grown by donor and receiver plants placed at a certain slope/ degree angle that allow normal flow of root exudates consist allelochemicals from donor to receiver plants, or the mesh thick barrier may be placed at a certain angle inside the pot that help gravity flow of root exudates.

Donor plants can be grown at different levels per pot of the same, higher or lower levels relative to receiver individuals and compare their effects. Different depths of sowing or placement of allelopathic materials could narrow/widen the interface area of the barrier between both species and thus speed/ delay the chemical interaction between species. Differeing the sowing depth of receiver species may allow judgment on the concentration and /or amount of allelochemicals produced with root exudates of donor plants, while small amounts or low concentration of allelochemicals may be adsorbed, modified, or diluted during passage in the soil and thus not possibly reach receiver species in enough toxic level. It is well known that plants inflicted by allelochemicals are growing close to donor species and in their surroundings. Increasing the distance depth between receiver and donor species could more eliminate any possible physical root overlapping of both species but increase probable chemical interaction. However, sowing depth is an important factor that insures germination and seedlings emergence.

Root space (restriction) of both can be controlled by increasing or decreasing the space allocated to each species and available for roots to occupy.

The technique allows testing the effect of intact living donor plants or dead plant tissues on or in the soil and their possible leached allelochemicals to receiver section. It also allows studying foliage leachates from intact living plants or dead plant tissues.

The technique allows studying the effect of donor plants on seeds as well as seedlings growth and development of receiver species and at different levels of growth factors and growth stage.

Donor plants and target species are cultured together in the soil under natural conditions

The effect of both competition and allelopathy in combination and of each separately can be determined.

The size and shape of pot section grown by donor or receiver plants can be varied and may be adjusted as needed to increase or decrease space occupied by any of the tested species.

Pore size and thickness of the metal-mesh barrier/ compartment may be chosen as required according to the root diameter of the tested species.

The technique can be also developed for use in field studies.

The barrier shape may be made as a small perforated basket that can be inserted inside a larger pot. The inner part (perforated basket) is grown by donor plants and the outer part by receiver species while roots of both are kept physically separated.

Discussion

Development of a real, accurate and convincing technique that simulate natural conditions and allows separation of allelopathy from competition is a real challenge to workers in the field of allelopathy. Although a large number of methods were proposed and many were implemented by different researchers worldwide, but careful inspection of these clearly show that all are also well suite competition studies. Weakness in the presently used allelopathy techniques may be applied to all followed in laboratory, glasshouse and field studies.

In spite of reported problems, a large number of publications on allelopathy were results of studies carried out under laboratory conditions using plant extracts tested on germinated seeds or against small sensitive seedlings which may not applicable to field conditions, while the seedling stage is the most vulnerable to high extract concentration, osmotic pressure or bound water-extract of the medium solution. This may be in part explaining the promotion effect of some extracts used at low concentrations and differences between extracts of different plant tissues or species. In the field, the inhibitory effect may not occur at seedlings stage, dissipate with time or disappear and transformed to stimulatory effect. Extraction (with cold or hot water or with organic solvents) destroys cells and can withdraw all plant cell contents. Extracts hardly represent the actual plant secretions (in time, types, chemical nature, forms, concentration and amounts) since not all released into the environment under natural conditions [23], allelochemicals are mostly expressed and released in response to stress factors [21,24] and act in minute amounts on target species not sensitive enough to detect by chemical techniques [25].

Different extracting methods, solvents (some of which are inhibitors) may change the nature of the extracted chemicals, plant/solvent ratios, dilutions, media, incubation temperatures, and many other variables are involved and may account for the effect obtained. Moreover, osmotic and/or matric potential of medium solution and seed coat and the effect of pH solution were in most cases not eliminated or determined.

Many of the identified chemicals were obtained either by extracting fresh or dried plants parts or isolated from the soil or soil solution. In all cases the isolated chemicals are not necessarily released into the environment from living plants or those detected in the soil may have been modified by microorganisms or not be available on time and concentration to receiver plants. Both lead to misinterpretation of allelopathy term, although isolated and identified chemicals may be of great value for industry and can be developed and synthesized as safe and effective bio-pesticides. However, unsuitability of laboratory bioassays in explaining field phenomenon is far long questionable and continuously criticized [4,5,26-28], since each was dependent upon the researcher's objectives, the

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target plants, the convenient conditions, and the availability of instruments. Therefore, all laboratory studies require proof in pot experiments or under field conditions to verify their ecological importance while opposite results may be obtained.

Glasshouse studies in pot experiments, hydroponics or circulated nutrient film technique (NFT) to detected allelopathy in root exudates on a target species have also many problems either in the techniques used or plant parameters measured.

Pot screening [17] without use of pre-germinated seeds may result in varied densities of both donor and receiver species, whereas allelopathic activity is density dependant [18,19]. Grinding plant tissues and their addition into potted soil may change soil structure, texture and compaction and thus soil water holding capacity, emergence and growth of the small seedlings or may exert high pressure on imbibed seeds ready to germinate. Regulation of allelopathy by soil factors such as soil texture, soil microbes, and soil chemical components were well documented [29-35].

Addition of plant materials as a percentage or ratio of the total soil volume/weight also create undetectable problems at which certain soil volume with its contents of nutrients is replaced by similar volume/weight of dried/fresh residues of the allelopathic species of lower or higher content or concentration of nutrients than that of the replaced soil portion. This creates a reduction gradient in the amount of nutrients available, increased with amount/volume of soil removed and is negatively correlated with the amount of plant tissues added. The uptake of nutrients by receiver plants may be affected if certain allelochemical molecule competed for the same carrying site in the root system leading to imbalanced uptake of nutrients from the soil. However, uptake of allelochemicals from the soil solution into the root system is not necessarily always a passive process and inhibitory molecules may be selectively absorbed by a target plant roots or in response to certain conditions. It has been reported that *Oryza sativa* seedlings grown under conditions of intense competition with *Echinochloa crus-galli* accumulated more allelochemicals than those grown in absence of competition [36]. Many of the techniques used in glasshouse studies do not simulate natural situation. It is well known that plants in water culture, hydroponics or grown in NFT system lack or of low developed root hairs in addition to other differences in the requirements, absorption, and translocation of mineral nutrients which may also account for the differences in results obtained [20]. Hydroponics and nutrient film techniques do not represent the natural situation and circulation of nutrients is irrelevant to the natural system of soil nutrient uptake and uses.

Many of the work carried out under laboratory or glasshouse conditions lack the standard methodologies determining the concentrations used or the volume of extract of plant materials or that added into the soil. Therefore different doses and extract volumes are still used and different plant tissues/solvent or soil/plant material ratios have been tested and reported with no standard methodology. However, all failed to isolate the effect of competition and/or physical effects of the materials examined on target species.

In the field and in different cases the applied allelopathic plant materials were harmful to both weed and crop plants or even more to crop plants [37,38] and many of the reported work failed to separate allelopathy from competition. Low density of *Cucurbita pepo* intercropped with *Zea mays* reduced weed biomass while high density was detrimental to both weeds and *Zea mays* [39]. Some or all benefits of soil mulch were eroded by phytotoxic leachates from residues of *Secale cereale* and *Trifolium subterranean* as cover crops [40]. Water soluble toxic substances of wheat straw mulching leached into the soil under natural conditions inhibited *Zea mays* growth and the effect was more pronounced under wet conditions [41] while wheat residues stimulated germination and growth of summer weeds [42].

Intercropping system ignored the complementary value of intercropped species and their differential responses and requirements for growth factors, while root exudates, or the symbiotic relationship, are other factors to be considered.

Cover crops may act through allelochemicals, competition or other mechanism including stimulation of microbes' allelochemicals, physical barriers, shading effects and changes in soil physical properties [43]. These are characterized by strong abilities to cover the soil surface and to effectively smother weeds [44]. Legumes covers elevate soil nitrogen level, availability and uptake. These crops are less competitive than others like cereals and crucifers. Soil mulch with living *Vicia villosa* has been reported to improve various soil physical properties including increase moisture, stabilization of soil temperature, increase water permeability and drainage and decrease soil hardness [45]. Changes in physical environment were responsible for the reduction in *Circium vulgare* seedlings emergence in the presence of leaf litter [46]. Good summer weed suppressions was achieved using *Mucuna prurience*, *Cortalaria juncea*, *Cortalaria spectabilis*, *Panicum maximum* and *Glyine max* as green manure while the effect was explained as due to the quick growth and good ground cover of these species [47].

Soil cover act by denying light, and prevention of photosynthesis. The ability of emerged seedlings to establish highly depends on thickness of the straw mulch layer.

All of the above mentioned factors should be taken into consideration dealing with role of allelopathy using cover crops for weed management.

Conclusion

The new developed technique has taken into considerations all difficulties encountered using presently and widely used techniques in glasshouse studies. However, possible modification and use of this technique is simple under laboratory conditions using intact living plants. It can separate allelopathy from competition with significant precision and may a count for differences in growth of receiver plants grown under both competition and allelopathy and each separately. It considered all factors involved using other techniques including role of density, special arrangement, growth stage,

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method of planting, role of environmental conditions, growth factors and stress conditions. It can be developed for use under field conditions and as such possible interference between competition and allelopathy can be eliminated. Root restriction of donor plants prevents root physical movement of these into receiver section and the only contact between roots of both is mainly through root exudates. However, root exudates consists a wide array of chemicals, organic substances including mineral nutrients and plant growth hormones and enzymes. Responses between different plant species to these are normally different while the toxic effects of allelochemicals depend mainly on dominance of allelchemical molecules in these exudates and differences on recovery of different plant species or their abilities to overcome their effects is widely different.

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