

## Abstracts of the 4<sup>th</sup> International Conference of Asian Allelopathy Society

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#### Insights into the mechanism of proliferation on the special microbes mediated by phenolic acids in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes

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*Radix pseudostellariae* L. belonging to the Caryophyllaceae family is a common and popular medicine in China, which is mainly produced in Fujian Province, Southeast of China. *R. pseudostellariae* contains ginseng saponins, polysaccharides, amino acids, flavonoids, and cyclic peptides, which can be used as a cure for spleen deficiency, anorexia, and palpitations. The average annual yield of *R. pseudostellariae* is about 5000 tons, accounting for more than 22 million dollars per year. However, this medicinal plants seriously suffer from the replanting disease in the popular monocropping system, in which the biomass and quality of root tuberous roots of the medicinal plants were decreased, indicating that the biomass of tuberous roots was declined by 33.3%, the polysaccharide content and ginseng saponins Rb1 in the tuberous roots were reduced by 88.08% and 44.33%, respectively. Therefore, it has become a top priority to elucidate the underlying mechanisms of replanting disease, especially in the case of medicinal plant production.

In this studies the phenolic acids, as potent allelochemicals, are believed to be associated with replanting disease and cause microflora shift and structural disorder in the rhizosphere soil of continuously monocultured R. pseudostellariae. Nine types of phenolic acids were identified both in the monocultured rhizosphere soil and in culture medium under sterile conditions. The author also found the phenolic acids could be transformed each other in the rhizosphere soil. Some special microorganisms utilized and catalyzed the conversion of an allelochemical vanillic acid into vanillin. Meanwhile, the K. sacchari was able to produce protocatechuic acid from consumption of vanillin, which then became an active agent in the allelopathy of understory species in the rhizosphere of R. pseudostellariae. The transcriptome sequencing was used to reveal the underlying mechanisms on the differential response of pathogenic bacterium Kosakonia sacchari and beneficial bacterium Bacillus pumilus to the allelopathic interactions of phenolic acids. Transcriptome analysis showed that vanillin enhanced glycolysis/gluconeogenesis, fatty acid biosynthesis, pentose phosphate, bacterial chemotaxis, flagellar assembly, and phosphotransferase system pathway in K. sacchari. However, protocatechuic acid, a metabolite produced by K. sacchari from vanillin, had negative effects on the citrate cycle and biosynthesis of novobiocin, phenylalanine, tyrosine, and tryptophan in *B. pumilus*. Concurrently, the protocatechuic acid decreased the biofilm formation of *B*. pumilus. These results unveiled the mechanisms how phenolic acids differentially mediate the shifts of microbial flora in rhizosphere soil, leading to the proliferation of pathogenic bacteria (i.e., K. sacchari) and the attenuation of beneficial bacteria (i.e., B. pumilus) under the monocropping system of R. pseudostellariae.

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### Using allelopathic approaches for ecological weed management in field crops

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Weeds pose a severe threat to food security for future generations. Chemical herbicides offer an effective control of weeds. However, continuous and indiscriminate use of herbicides may cause of development of herbicide resistant weed biotypes and environmental pollution. In this regard, allelopathy offers attractive and eco-friendly option for ecological weed control. Allelopathy can be utilized, for weed control, through inclusion of allelopathic crops in rotation, use of allelopathic mulches, intercropping of allelopathic crops and use of allelopathic plant extracts. In a series of studies, we noted that inclusion of sorghum in rotation with maize, wheat and rice caused substantial reduction in weed infestation in conventional and conservation crop production systems. Application of sorghum mulch, use of sorghum as intercrop, and exogenous use of sorghum water extract also suppressed the weeds. However, combination of more than one approach was more effective than the single strategy. In this regard, inclusion of sorghum in rotation together with sorghum mulching (8 t ha<sup>-1</sup>) and exogenous application of sorghum water extract (15 ha<sup>-1</sup>) was more effective in suppressing weeds in maize, rice and wheat, which was then translated in increase in yield and profitability. In conclusion, integrated use of allelopathic approaches offers cost-effective option for non-chemical weed management in conventional and conservation maize, rice and wheat production systems.

# Allelopathy and allelochemicals between phytophagous insets and plants

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The term "allelopathy" is defined as an interactive phenomenon between different species. This is not only a plant-plant interaction, but also includes an insect-plant and microbial-plant interaction. Most insect tolerant plants imply any physical and chemical defense system against their natural enemies. One of the physical defense system is having a hairy leaves and/or thorns on their plant surface. On the others hands, the chemical defense system is production and storage of the insect toxic secondary metabolites. Especially, wild plants without protection of pesticides and cultivars closed to its native species maybe maintain the defense system against their natural enemy. Based on this, many insect antifeedants were isolated from various plants, Asteraceae, Orobancaceae and Cyperaceae. And the almost isolated natural products were allelochemicals, since these chemicals existed in/on the plant and the phytophagous insect will be directly contact with them when they fed to the plant.

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### Branched broomrape (*Orobanche ramosa* L.) control in tomato (*Lycopersicon esculentum* Mill.) by trap and other plant species in rotation

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Forty-four plant species of 13 families were tested as traps reducing Orobanche ramosa L. infestation on tomato under glasshouse conditions. Many species were parasitized but Orobanche shoot number and dry weight were greatly varied. Trifolium alexandrinum was most infested and Citrullus vulgaris least. Tomato growth was reduced after many tested species while none prevented parasite infection. Parasite shoot number was highest on tomato followed Anethum graveolens, Coriandrum sativum, Cucumis melo var. flexuosus, T. alexandrinum, Sesamum indicum, and Solanum elaeagnifolium, and lowest after Brassica oleracea var. italic, Brassica rapa var. rapa, Capsicum annuum, Cicer arietinum. Citrullus colocynthis, Cuminum cyminum, Capsicum Cucurbita maxima, Hordeum vulgare, Linum usitatissimum, frutescens, Vigna sinensis. Spinacea oleracea cv. Epinard greant and Parasite dry weight was lowest on tomato followed C. frutescens, C. melo, Trigonella foenum-graecum, Hibiscus sabdariffa and Pimpinella anisum but highest after Oroganum syriacum and C. colocynthis. Many reported Orobanche traps were parasitized. Tomato growth was doubled following T. alexandrinum and Ecballium elaterium. Nonhost species reduced O. ramosa on tomato may be regarded as parasite traps. The best tomato growth and parasite control were obtained after L. usitatissimum and V. sinensis followed by C. sativum, E. elaterium, H. sabdariffa, H. vulgare, S. indicum and Sorghum vulgare.

Keywords: Parasitic weed, Branched broomrape, *Orobanche ramosa*, Tomato, Traps species, Catch species, Control, Rotation.

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# Protoplast co-culture method with digital image analysis for bioassay of allelopathy

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'Protoplasts Co-culture Method' was developed to study allelopathic activities of plants using tissues and their cultured cells. This method is based on the idea of co-culturing of callus of a test plant to determine its allelopathic effects on the growth of another callus. However, quantitative evaluation of experimental results is difficult using the callus co-culturing method. Therefore, we developed a protoplast co-culture method. The response of cultured protoplasts of a recipient plant can be expressed as numbers of non-spherically enlarged, divided protoplasts, in a 50 µL of medium in a well of a 96-well culture plate. For example, inhibitory effects of protoplasts of a test plant, on growth of recipient protoplasts of lettuce were determined in a Murashige and Skoog's medium containing 2,4-dichlorophenoxyacetic acid and benzyladenine, sucrose and osmoticum mannitol. Protoplasts densities were varied. The results were in agreement with those obtained by the conventional in vitro bioassay method and field tests. Not only lettuce but also other plants, e.g. rice can be used as recipient plant species. These results will contribute to clarify the cellular mechanism(s) of allelopathy. Furthermore, we also found an accumulation of a yellow color (carotenoid) after cell wall formation and divisions of lettuce protoplasts. After scanning of 96-well culture plates, the inhibition of yellow color accumulation was quantified by the Image J analysis. As lettuce is widely used as a recipient plant in conventional in vitro bioassay methods, this simple high-throughput method (DIA-PP method) will make possible to survey effects of a large numbers of test plants and contribute to the discovery of new allelochemicals.

### Control of Panama disease of banana by intercropping with Chinese chive: Role of allelochemcals

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Panama disease of banana caused by *Fusarium oxysporum* f.sp. cubense (FOC) is a devastating soil-borne fungal disease causing extensive failure of commercial plantation of banana throughout banana producing areas of the world. The pathogen is extremely difficult to control due to its long persistence in soil, and to date no effective control measure has been found. Our study showed that intercropping banana with Chinese chive (Allium tuberosum Rottler) could control the Panama disease. Both volatiles and water extracts of Chinese chive suppressed mycelial growth of FOC. Three chemicals including dimethyl trisulfide (DMT), dipropyl trisulfide (DPT) and 2-methyl-2-pentena (MP) isolated from Chinese chive strongly inhibited mycelial growth and spore germination of FOC. Different varieties varied with their inhibitory effects. Volatile compounds emitted from the intact growing roots mimicking natural environment inhibited spore germination of FOC. We identified five volatiles including 2-methyl-2-pentenal and four organosulfur compounds (dimethyl trisulfide, dimethyl disulfide, dipropyl disulfide, and dipropyl trisulfide) from the leaves and roots of Chinese chive. All these compounds exhibited inhibitory effects on FOC, but 2-methyl-2-pentenal and dimethyl trisulfide showed stronger inhibition than the other three compounds. Our results show that antifungal allelochemicals released from Chinese chive help control Panama disease in banana. We conclude that intercropping banana with Chinese chive can control Panama disease and increase cropland biodiversity.

Key words: Fusarium wilt ; Panama disease of banana ; Intercropping ; Chinese chives ; Ecological control

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# Allelopathic extractions improve growth and yield of wheat

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Allelopathy is an eco-friendly science emerged in the past few decades. Allelopathic plants suppress or promote the growth of other plants. The allelopathic plants posses several secondary metabolites which are highly water soluble. These can easily be extracted by soaking dry herbage in water. We have conducted series of field experiments at farmers fields and research stations. Results of the trails have shown considerable improvement in growth of crops. The water extracts of brinjal, garlic and sorghum (3%) applied at tillering and earing, enhanced the yield of wheat by 10 to 40%. In conclusion use of alleolpathic extracts at low rates (3%) is highly economical and viable for farmers. Results of some of the recent experiments will be discussed.

Key words: Allelopathy, Garlic, Brinjal, Sorghum, Wheat.

### Characterization of plant growth-promoting effect of γ-terpinene and β-caryophyllene in lettuce and maize seedlings

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In our previous studies, screening of volatile compounds having plant growthpromoting activity was performed using various plant species, and we found that  $\gamma$ -terpinene and  $\beta$ -caryophyllene showed growth-promoting activity in lettuce roots. In this study, the growth-promoting effects of  $\gamma$ -terpinene and  $\beta$ caryophyllene on root and shoot of lettuce and maize seedlings were investigated. Root fresh weight increased up to 130% of control at 138 µmol/L and 150% at 518  $\mu$ mol/L in maize seedlings 3 days after treatment with  $\gamma$ terpinene and  $\beta$ -caryophyllene, respectively. On the other hand,  $\beta$ -caryophyllene reduced the fresh weight of maize hypocotyl by 70% of control 3 days after treatment, whereas  $\gamma$ -terpinene had no effect on the hypocotyl fresh weight. In lettuce seedlings, 357  $\mu$ mol/L of  $\gamma$ -terpinene stimulated root length and third leaf area up to 120% and 140% of control 14 days after treatment, respectively.  $\gamma$ -Terpinene (0–357  $\mu$ mol/L) also increased the number of lateral root in a concentration-dependent manner in lettuce. Similarly, β-caryophyllene stimulated root length of lettuce up to 150% of control 14 days after treatment, but inhibited the third leaf area by 50% of control at 50 µmol/L. Overall, the growth-promoting effects of  $\beta$ -caryophyllene were more significant than that of  $\gamma$ -terpinene. In proteomic analysis,  $\gamma$ -terpinene increased the abundances of 70 proteins including IAA-amino acid hydrolase and xyloglucan endotransglucosylase/hydrolase (XTH) in lettuce roots. From these results, auxin and XTH might be involved in the growth-promoting effect of  $\gamma$ -terpinene in lettuce roots.

### Involvement of ROS and xyloglucan endotransglycosylase/hydrolase in S-(+)-carvone-induced growth inhibition in Arabidopsis thaliana seedlings

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(S)-(+)-2-Methyl-5-(1-methylethenyl)-2-cyclohexenone (S-(+)-carvone) is a bioactive volatile terpeniod produced by Aloysia polystachya and it shows strong growth inhibitory effects on several plant speices. However, its mode of action is not yet understood. The present study was conducted to investigate the mechanism of growth inhibition of S-(+)-carvone in Arabidopsis thaliana seedlings. Shoot and root growth of A. thaliana were inhibited by S-(+)-carvone  $(0-527.6 \ \mu mol/L)$  in a dose-dependent manner. S-(+)-Carvone increased the generation of reactive oxygen species (ROS) in shoots and roots at concentrations >255.6  $\mu$ mol/L and > 114.2  $\mu$ mol/L 5 days after treatment, respectively. This compound also caused lipid peroxidation in shoots and programed cell death in roots at concentrations >255.6 µmol/L and >527.6  $\mu$ mol/L 5 days after treatment, respectively. Furthermore, S-(+)-carvoneinduced root growth inhibition was reduced by application of ROS scavenger, ascorbic acid or 1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine. In qRT-PCR analysis, S-(+)-carvone (255.6 µmol/L) markedly suppressed transcription of several xyloglucan endotransglycosylase/hydrolase (XTH) genes in roots of A. *thaliana* 24 hours after treatment. From these results, primary action of S(+)carvone to inhibit growth of A. thaliana may be caused by ROS overproduction and subsequent oxidative damage. However, additional studies are required to elucidate the involvement of XTH gene suppression in S-(+)-carvone-induced plant growth inhibition.

### The existence of functional substances released from mycorrhizal fungi for the germination of *Spiranthes sinensis*. var. *amoena*

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There are many symbiotic species in plants and microbes. It is well known that mycorrhizae are fungi living inside plants. The seed germination of orchid plants depends strongly on the existence of mycorrhizae. We have investigated the relationships between an orchid, *Spiranthes sinensis* var. *amoena*, and a mycorrhizal fungus, *Epulorhiza repens*, during their germination process. There are several steps during seed germination. The first step is the swelling of seeds. In this study, swelling activity was investigated as the first step of their germination.

All of the plant samples were collected from the campus of University of Tsukuba. We have already established a method for calculating the biomass of a mycorrhizal fungus growing in *Spiranthes sinensis* var. *amoena* in our laboratory. *Epulorhiza repens* was collected by Mr. Fujimori and purified as a strain *Epulorhiza repens* T338. *Epulorhiza repens* T338 stored at our laboratory was used as symbiotic fungus species. The seeds were too small to count individually, so, the correlation of the wight and their seed number was investigated. After sterilization with sodium chloride, seeds were put on the surface of medium with and without the fungus. The seed germination was observed by microscopes and the rate of germination was calculated.

Our results indicate that some functional substances exist in the medium released from grown fungi. We will show the results and other recent our study related to Orchids plants and thier symbiotic fungi.

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### Determination of allelopathic potentialities of Bangladeshi plant species

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Competitive relationships of crops and weeds are the major problems in the field culture which reduces gross crop production every year in the South Asian country, Bangladesh. Excessive use of synthetic fertilizers and herbicides may create a resistant feature to the weeds and ultimate sickness of soil and farmers' health. From the perception of Allelopathy, we are piercing natural chemical as a secondary metabolite that might reduce the aggressiveness of native weeds including invasive plant preserving agricultural sustainability. We manipulated sandwich (SW) method developed by Fujii *et al.* (2003). Lettuce growth in SW was observed to know the influence of leaching the phytochemical from the different parts of the plants as allelochemical from these plant species. We screened 87% allelopathic plant species among the 215 plant species. Where 37% species shown higher inhibitory (HI), followed by 21% species, moderate inhibitory (MI) activities under the considerable standard 10 mg in lettuce radical length.

From this study, we found among 37%, HI species *Acacia concinna*, *Couroupita guianensis*, *Piper longum*, *Aegle marmelos* and followed by *Azadirachta indica*, *Sapindus mukorossi* caused strong inhibition on lettuce radicle growth. Among the screened plant species, Shikakai (*Acacia concinna*), family Fabaceae, followed by Cannonball tree (*Couroupita guianensis*), Lecythidaceae showed a strong allelopathic activity. Advance studies about the selective plants to identify the potential allelochemical would be considerable. As a concern of weed management, these allelopathic plants are the significant potential alternatives relate to eco-friendly crop culture system.

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### Carnosic acid; the Principal Plant Growth Inhibitor in *Rosmarinus officinalis* Leaves

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Weeds are rapidly developing resistance to synthetic herbicides and exploring allelopathic species can help minimize the ecological threat posed by herbicideresistant weeds. In this study, we aimed to evaluate the contribution of some bioactive compounds in rosemary (*Rosmarinus officinalis*) to its allelopathy based on the total activity approach. The phytotoxic activity of crude extracts of rosemary plant parts (leaves, roots, inflorescences, and stems) was tested on receptor *Lactuca sativa*. Soils incorporated with rosemary dried leaves were also tested on test plants. The various parts of rosemary were evaluated for the content of some bioactive compounds (caffeic, ferulic, gallic, rosmarinic, carnosic acid, and chlorogenic acids) by reversed-phase HPLC. The specific activity and total activity of crude extract and individual compound were evaluated using lettuce as test plant.

We found that the growth inhibitory effect of the crude extracts of the rosemary leaves was the highest among all parts. Dry matter and seed emergence of lettuce were inhibited by soil amended with rosemary leaf debris. Carnosic acid was the main compound found in rosemary leaves and also had a high specific activity. The inhibitory activity of the rosemary leaf extract could be explained by the high presence of carnosic acid. Hence, we assume based on the total activity estimation that carnosic acid among the other tested compounds is the major allelochemical in rosemary leaves

### Effects of Garlic Allelochemical Diallyl Disulfide on Tomato and Its Mechanism in Alleviating Continuous Cropping Obstacle

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In recent years, the continuous cropping obstacle has become the restricting factor for the sustainable development of facility vegetables. In our previous studies, we found that continuous cropping obstacle of tomato (Solanum lycopersicum L.) could be alleviated by relay intercropping with garlic (Allium sativum L.), and the allelopathy of garlic root exudates may play a major role. Diallyl disulfide (DADS) is one of the main allelochemicals in garlic root exudates and garlic straw, however, the physiological and molecular mechanism of DADS on tomato are still unclear. The clarification of physiological and molecular mechanism of DADS-promoting effect on tomato plant and its alleviating effect on obstacles to continuous cropping of tomato have important significance in the sustainable production of greenhouse tomato.

The objectives of this research were: to study the physiological and biochemical effects of DADS on tomato seeds germination and seedling growth; to investigate the effect of DADS on the expression of tomato root genes, analyse the sensitive metabolic pathways and functions of major genes which response to DADS; to test the allelopathic effect of DADS on tomato plant growth under continuous cropping conditions and analyze the impact of DADS on tomato root growth at low concentrations (0.01-0.62 mM) but inhibited root growth at high concentrations (6.20-20.67 mM); 2. DADS may activate tomato resistance in three ways: increasing the contents of nonenzymatic antioxidants, such as GSH; inducing the activity of enzymatic antioxidants, including SOD, POD, CAT, PPO and GR; and activating ETI; 3. DADS may change the synthesis of plant hormones and signal transduction, thereby influencing tomato plant growth and resistance; 4. DADS may change the content of tomato root exudates to reduce autotoxicity and increase the tomato disease resistance.

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### Identification and allelopathic mechanism of green garlic volatiles on cucumber seedlings

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Plants synthesize and emit a large variety of volatile organic compounds (VOC) to interact with other organisms. Garlic and medicine made of garlic volatiles are increasingly being used as fungicides in modern agriculture. However, the component and effect of garlic volatiles in crop plants have been less studied. Here, green garlic volatiles was chose to explore the questions. First, it was collected by headspace solid-phase microextraction (HSPM) method and analyzed by gas chromatography-mass spectrometry (GS-MS). The results showed that diallyl disulfide (DADS) is the main allelochemical compound of green garlic volatiles. Here, we exposed 2 true leaf-stage cucumber plants cultivated in artificial media to different concentrations of a garlic (0, 6 12 and 18 bulbs) and DADS (0 and 1 mM) for 10 d. On this basis, the allelopathic mechanism of green garlic volatiles in vivo and DADS on the cucumber (CCMC) seedlings growth and physiology was further studied. In summary, they can promote the growth of cucumber seedlings and increase the antioxidant enzyme activities and the accumulation of reactive oxygen species ultimately.

### Effect of Momilactone B on Seed Germination and Root Development of *Arabidopsis thaliana* and the Related Mechanisms

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Momilactones are diterpenoid phytoalexins produced by rice (Oryza sativa L.). Momilactone B (MB) is the key allelochemical of rice allelopathy and one of most phytotoxic natural products. However, the mode of action MB is unknown. In this study, Arabidopsis thaliana was used as a receiver plant to dissect the mechanism of allelopathic effect of MB. A dose-dependent inhibitory effect on seed germination and seedling growth of Arabidopsis were observed. Seed treatment with MB led to up-regulation of ABA synthesis genes and ABA signaling pathway genes, as well as strong induction of negative regulation genes in GA signaling pathway. Furthermore, Arabidopsis thaliana mutant abi4 showed less susceptible to MB. Our results indicate that inhibitory effect of MB on seed germination results from suppression of GA signal pathway and activation of ABA signal pathway. Momilactone B inhibited the length of main roots and the number of lateral roots. Confocal microscopy observation showed that the length of apical meristem was significantly shorter and the number of cells was significantly decreased after MB treatment, suggesting that MB inhibits apical meristem and cell division. Exogenous application of 10 mM GA3 or 0.1 nM NAA alleviated inhibitory effect of MB on main roots and lateral roots. In conclusion, MB inhibited root growth of Arabidopsis by inhibiting auxin transportation and reducing auxin accumulation at the root tip, as well as the activity of root apical meristem and cell elongation. Our results suggest that MB inhibits Arabidopsis by disturbing hormone signaling transduction.

Key words: Momilactone B; seed germination; root development; rice allelopathy

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## 0-9

## Allelopathy in context of resolution at ecosystem level

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The molecular interactions by which the plants affect their neighboring species either at the species or ecosystem level is attributed to allelopathy consequently affecting the floral populations and communities. Recent evolvement in assessment of the bioassays made possible the examination of the plant/vegetation interaction at molecular level. In this case we have made the comparison of allelopathic interactions in two different ecosystems instead of population/community level of resolution thus hypothesizing that allelopathy is influencing the ecological communities in a more obvious ways. In this study, as an initial approach impact of herbivory by wildlife harboring the particular ecosystem is considered which reflects the allelopathic potential of plant species as their defense mechanism thus altering or affecting the vegetation in the long run. This is illustrated by the contrasting examples from Karakoram-West Tibetan Plateau alpine steppe and Potohar Plateau. The earlier area is considered as roof of the world and presents a mix of Mountain Sub-Tropical Scrub Forests, Mountain Dry Temperate Coniferous Forests, Mountain Dry Temperate Broadleaved Forests and Northern Dry Scrub Forests. However, Potohar Plateau presents the sub-tropical broad leaved evergreen forest ecosystem. We have assessed how two exotic species has affected the respective ecosystem properties by producing the secondary metabolites i.e. Ailanthus spp. and Prosopis cineraria. In both cases, the production of metabolites has regulated the key ecosystem processes thus determine the ecosystem functionality and consequently the structure of vegetation community in respective areas. Our study supports the hypothesis that allelopathy is more obvious and operational at ecosystem level of resolution, instead of species level of resolution.

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### Management of charcoal rot of black gram by soil amendment with *Ageratum conyzoides*

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Black gram [Vigna mungo (L.) Hepper] is an important pulse widely used in Pakistan. Its yield is significantly reduced by charcoal rot disease caused by Macrophomina phaseolina. The present study was carried out to assess the disease management potential of dry biomass of an asteraceous weed Ageratum conyzoides used as soil amendment. Pot soil was made sick with M. phaseolina inoculum and after seven days, dry biomass of the weed was mixed in pot soil at 0.5, 1, ..., 3% (w/w). Treatments included a negative control, a positive control (fungal inoculation alone), and fungal pathogen in combination with different quantities of dry biomass of A. convzoides. Seeds of black gram were sown in these pots at 10 seeds per pot and the crop was harvested at maturity. Data regarding disease incidence, shoot and root biomass, and grain yield were recorded and analyzed statistically by applying Tukey's HSD test at 5% level of significance. Activities of defence related enzymes viz. peroxidase (PO) and phenylalanine ammonia lyase (PAL) were noted at flowering stage. The highest disease incidence (100%) was recorded in positive control that was gradually decreased with increase in A. conyzoides dry biomass in the pot soil. A 2% soil amendment completely controlled the disease. The highest grain yield (5.8 g  $pot^{-1}$ ) was recorded in 2.5% soil amendment as compared to 1.43 g  $pot^{-1}$  in negative and 0.132 g pot<sup>-1</sup> in positive control treatment. The highest PAL and PO activities were recorded in positive control. These activities were gradually decreased with increase in dry biomass of the weed in the soil.

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# 0-11

# Allelopathic water extracts helps improve drought resistance during reproductive stages in wheat

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This experiment was conducted in pots where two wheat cultivars viz., Mairaj-2008 (M-2008) and Faisalabad-2008 (FD-2008) were grown in the pots filled with 10 kg of the soil. The pots were irrigated as per need of the crop until leaf boot stage at 75% WHC which was followed by imposition of drought stress at reproductive (booting and anthesis) and grain filling (dough stage) stages in both wheat cultivars. When one week passed away after foliage application of water extracts on sorghum, brassica, moringa and sunflower, the drought was imposed at each stage by maintaining the pots at 35% WHC. Drought stress negatively altered performance of wheat crop in terms of morphology and yield; however, the yield of wheat under drought was improved after the foliage application of allelopathic water extracts. M-2008 performed better than FD-2008 under drought stress which was visible through improvement in the performance of this cultivar under terminal drought stress. Although, application of all the AWEs improved the performance of both cultivars, the water extract of sorghum was most useful under drought stress than other extract. Drought stress imposition at booting and anthesis was more injurious to crop than when imposed at grain filling. In crux, foliage applied AWEs applied alone or in combination might be pragmatic option to improve wheat performance under climate change.

Keywords: drought, wheat, morphology, climate change, water extract

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### Combined application of natural plant water extracts and biochar improves the productivity of bread wheat

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Bread wheat (Triticum aestivum L.) is staple of Pakistani people. However, its yield at farmer field is low as compared with its genetic potential. Integration of various crop and soil management strategies might be an option to enhance wheat productivity at farmer field. This 2-year experiment was conducted to check the influence of combine application of natural plant water extracts and biochar on the productity of wheat during the winter season of 2015-16. The experiment consisted of seven treatment viz. (1) control (2) application of biochar (0.18 kg pot<sup>-1</sup>) alone, (3) application of sorghum water extract (SWE) alone, (4) application of moringa water extract (MWE) alone, (5) application of biochar + SWE, (6) application of biochar + MWE, (7) application of biochar + SWE+MWE. The results revealed that application of both crop water extracts in combination with biochar improved the growth and grain yield of wheat. Use of MWE in combination with biochar enhanced the grains per spike, 1000-grain weight and grain yield of wheat by 44, 14, and 24%, respectively than the control treatment. In crux, use of MWE in combination with biochar might be a viable option to improve the productvity of bread wheat.

Key words: bread wheat, biochar, grain yield, natural water extracts

# 0-13

### Allelopathic potential of African marigold (*Tagetas erecta*) for managing root knot nematode (*Meloidogyne incognita*) in tomato

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Root knot nematode (*Meloidogyne incognita*) pose a significant threat to crop production in Pakistan which causes a wide range of losses to agricultural commodities such as delayed maturity, toppling, reduced yield & quality, high costs of production consequently loss of income. This study was carried out to check the effect of African marigold (Tagetes erecta) to control the root- knot nematodes (RKN) infesting tomato (Solanum lycopersicum L.) roots. The treatments included tomato plants (1,2), marigold plants (0,1,2) and RKN inoculation (0,1) arranged in various combinations in pots. The results showed that maximum plant height (79.14cm), number of leaves (28.00), leaf area (24.46cm<sup>2</sup>), number of fruit plant<sup>-1</sup> (42.66), fruit Size (44.26cm<sup>2</sup>) of tomato after 45 days of transplantation were observed in the treatment having two plants of marigold, one plant of tomato along with the inoculum of RKN as compared to other intercropping treatments and control. Pot soil was analyzed for NPK, EC and pH before and after transplanting. Significant differences were observed for NPK. It was observed that intercropping marigold was beneficial in controlling nematode populations to greater depths by altering soil pH moderately acidic (8.01) with high electrical conductivity (0.14). Fruit quality attributes like firmness (4.86Nmm<sup>-1</sup>), TSS (4.9°Brix) and shelf life (8.67days) were best in intercropping treatment comprising two plants of marigold with one plant of tomato having an inoculum of RKN in a single pot. The results may be attributed due to release of allelochemicals by Marigold in the surrounding environment that inhibited the activity of Root knot nematode, which had a positive effect on growth and development of tomato accompanied by significant enhancement in yield. It may be concluded that plant parasitic nematodes may be controlled through intercropping tomato with marigold, which is a safer alternative to traditional application of agrochemicals for disease management.

### The role of negatively indirect allelopathy in tea consecutively monoculture ratooning problems

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Long-term consecutive monoculture of crops leads to decreased growth and yield in following years, due to allelopathy, the key culprit. In long-term consecutive monoculture tea orchards, plant performance such as growth, quality and yield decreases over time after the establishment of tea plants in the same soil for several years. Therefore, addressing allelopathic mechanisms of leaves litters by falling and pruning every year in long-term monoculture tea orchards are critical to tea industry. To help characterize the allelopathic mechanism of tea, we carried out a large-scale, pyrosequencing analysis of the tea rhizo-compartments microbiomes in response to the stress of catechins, well-known allelochemicals from tea leaves litters. Our main result showed that, pH of tea orchard was significantly lowered with increasing planting age. Furthermore, long-term tea cultivation resulted in accumulation of allelochemicals in the rhizosphere including Epigallocatechin Gallate (EGCG), Epigallocatechin (EGC), Epicatechin gallate (ECG), Catechin (±C), Epicatechin (EC) in the form of leaves litters. Meanwhile, phosphomonoesterase, sucrase and urease activity were significantly declined. However, the activities of catalase and polyphenol oxidase were highly increased in long-term 20-30-years consecutively monocultured tea field. Moreover, distinct distribution patterns of catechins degrading bacterial taxa were analsed in all three rhizo-compartments (i.e., rhizosphere, rhizoplane and endorhizosphere) of 30-year monoculture tea mediated strongly by soil pH and catechin containing compounds. Pyrosequencing results identified Pseudomonas and Burkholderia as the dominant groups at genus level while plant growth promoting bacteria such as Bacillus, Sphingomonas, and Prevotella were significantly lowered in long term monoculture degraded tea plantation. The qPCR results in situ also confirmed that the whole bacterial contents in per gram degraded 30-year tea plants soil was significantly lowered as compared to fresh soil tea plantations. However, Pseudomonas population was significantly increased with increasing tea plantation years. In-vitro study indicated that with increasing the concentration of these allelochemicals, the growth of catechin degrading bacteria such as Pseudomonas was increased but reverse was true in the case of the growth promoting bacteria such as Bacillus. Furthermore, invitro-interaction results showed that the Pseudomonas genera are involved in the degradation of catechins into more phytotoxic allelochemicals such as EC and PCA. The pH of the broth with the accumulation of PCA was also decreased to 0.36-fold after 72 hours. Therefore it can be concluded that the shift of beneficial microbes and the accumulation of active allelochemicals may be the cause of acidification, soil sickness and regeneration problem which may attribute to hindrance of plant growth, development and low yield in 30-year long-term monocultured tea plantation. These results helped us to clarify the role of leaves litters contamination in long-term continuously rationed and monocultured tea orchard that can inevitably lead to soil acidification and fertility decline, in turn resulted in decresed yield and quality of tea.

**Keywords**: Monoculture; Allelochemicals; Microbiomes; Rhizo-compartments; High-throughput sequencing; Redundancy analysis (RDA); High performance liquid (HPLC), Chromatography-Electrospray Ionization-Mass spectrometry (HPLC-ESI-MS), rhizosphere restoration, organic agriculture

### Identification of antifungal constituents from Agaricus bisporus (J.E. Lange) Imbach

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In agriculture, fungal invasions cause heavy loss and affect food security and food safety. Chemical pesticides are available to control these pathogens but these are associated with environmental and public health concerns. In recent years, there is growing trend towards nature friendly methods of pathogen control. Use of allelochemicals from mushrooms is a promising fileld. In the present study, antifungal activity of an edible mushroom, Agaricus bisporus was investigated. For this purpose, different organic solvent extracts of A. bisporus, were evaluated against plant pathogenic fungal species viz. Macrophomina phaseolina, Alternaria alternata, Fusarium oxysporum, Drechslera australiensis and Aspergillus flavus. To check the antifungal activity of A. bisporus, serial dilution method was used. Six levels of treatments of each *n*-hexane, chloroform and ethyl acetate (0, 5, 10, 15, 20 and 25 mg/ml) were applied against all fungal species. Generally, all organic solvent extracts reduced the fungal biomass significantly with the increase in concentration but, ethyl acetate fraction exhibited better results and reduced the fungal growth in some species up to 50% like D. australiensis and 44% each in A. alternata and F. oxysporum. From GCMS analysis of ethyl acetate fraction, 10 compounds were identified. Out of these 10 compounds, only one compound [1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester], molecular formula=  $C_{16}H_{22}O_4$  and molecular weight= 278, showed 91.31% peak value. It was concluded that this compound being in the highest concentration in the ethyl acetate fraction of A. bisporus mushroom is responsible for its antifungal activity, recorded in the present study. The present investigation concluded that A. bisporus has bioactive compounds that can be exploited to develop ecofriendly fungicides against a number of plant pathogens.

### Change on the allelopathy and rhizospheric soil microbial diversity by regulating *OsMYB57* expression on rice

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Allelochemicals are mainly produced as plant secondary metabolites. MYB transcription factors are documented that effectively regulate plant secondary metabolic pathways, which suggests a potential role of MYB in rice allelopathic agents. The study here showed that transcription factor OsMYB57 (LOC Os02g40530) positively regulates rice allelopathic effect on barnyardgrass. The gene expression level of O-methyltransferase and cinnamoyl coenzyme A were up-regulated in the OsMYB57 transcriptional enhanced rice, whilst they were down-regulated in the OsMYB57 transcriptional repressed rice, compared to wild-type rice. Enhancement of OsMYB57 expression also led to increased microbial populations in the rhizosphere of rice. Among the microbes, Bacillus spp., Pseudomonas spp., and Streptomyces spp. were observed suppressing the barnyardgrass growth with higher inhibitory rate, and their populations were highest in the rhizospheric soil of OsMYB57-enhanced rice. A further study indicated that the phenolic acids, including p-hydroxybenzoic acid, cinnamic acid, and ferulic acid were able to facilitate the growth of Bacillus subtilis. Streptomyces and Pseudomonas spp., revealing the interactions between these microbes and phenolic acids. Our results demonstrated that transcription factor OsMYB57 functions in modulation of the rice allelopathic potential by regulating the genes from phenylpropanoid pathway. The allelochemicals secreted by rice roots promoted the gathering of microorganisms, and the joint-interactions between allelochemicals and particular microbes dominate the allelopathic effect on target barnyardgrass.

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### Influence of *Trichoderma harzianum* in growth, plant defense-related genes and metabolites of *Radix pseudostellariae*

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Consecutive monoculture of *Radix pseudostellariae* in the same field results in serious decrease in both yield and quality. Many *Trichoderma* species are reported to be antagonistic towards plant pathogen. In this study, we study how the interaction of plant with *Fusarium oxyproum* and *Trichoderma* affect the plant's growth. A three-year field experiment was used to explore the shifts in the structure and diversity of rhizosphere *Trichoderma* communities by DGGE. Bioassays were performed in the fields, and yield of the plants was evaluated. The effect of *Trichoderma* treatment on the expression of *R. pseudostellariae* defense-related and pathogenesis-related genes was analyzed by real-time PCR. In addition, we also analyzed the root samples of *R. pseudostellariae* treated with *T. harzianum* ZC51, based on metabonomics.

The results demonstrated that extended monoculture significantly declined the diversity of Trichoderm spp. Quantitative PCR analysis showed a significant decrease in Trichoderma spp, especially in T. harzianum. Furthermore, a strain of T. harzianum ZC51 antagonistic to F. oxyproum was isolated from rhizosphere soil. Plant biomass showed that T. harzianum ZC51 performed good and protected R. pseudostellariae from infection by F. oxysporum, and the yield of R. pseudostellariae which was treated with T. harzianum ZC51 could reach 77.04% of the newly plant (FP). Moreover, the interaction of plants with T. harzianum ZC51 affects the level of expression of two defense-related genes, and three pathogenesis-related genes. In addition, metabonomics results show that 18 kinds of mutual metabolites with significant differences, including amino acids, sugars, organic acids, and organic compounds. In conclusion, this study demonstrated that extended monoculture of R. pseudostellariae could alter the Trichoderma communities in the plant rhizosphere, leading to relatively low level of antagonistic microorganisms. But TR treatment could enhance the expression of defense-related genes, and the yield of R. pseudostellariae.

### Features of microbiomes in the rhizosphere soils of Achyranthes bidentata tolerant to consecutive monoculture

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In the long-term practice, it was found that most medicinal plants suffered from serious replanting diseases. Interestingly, in case of A. bidentata it is opposite to the aforementioned and a very suitable plant species for continuous monoculture. Therefore, in this study, the rhizosphere soils which was grown in a monoculture for multiple years were investigated for analyzing the rhizosphere microbial community structure by means of high-throughput 16S rDNA sequencing and denaturing gradient gel electrophoresis (DGGE) techniques. The results showed that there was significant enhancement in the abundance of some beneficial species such as Pseudomonas spp and Bacillus spp. The DGGE profiles revealed that the abundance of Pseudomonas aeruginosa (P. aeruginosa) was distinctly higher in consecutively monocultured plots than in the newly planted plot. Further, in the exogenous addition experiment showed that: A strain of P. aeruginosa W39 from our lab was found to have plant growth promoting activities, such as build-up of antagonistic effect, production of auxin and increase in biomass of A. bidentata (P<0.5). The results of the study suggest that one of the chief reasons for A. bidentata tolerance to continuous cropping may be the increase in the number of beneficial bacteria mediated by root exudates in the rhizosphere soil with the increasing years of continuous cropping.

### Comparative metagenomics analysis of soil microbial communities under *Rehmannia glutinosa* consecutive monoculture

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Rehmannia glutinosa, a perennial herbaceous plant belonging to the family Scrophulariaceae, is highly valued in traditional Chinese medicine. However, this plant suffer from severe allelopathic autotoxicity, also known as replant disease or consecutive monoculture problem in the agricultural production. Consecutive monoculture of this plant in the same field results in very serious decline in both quality and yield of tuberous roots, with poor field performance and insufficient resistance. The root-associated microbiome, referred to as the second genome of the plant, is closely associated with the soil health and plant performance. In this study, pyrosequencing of 16S rDNA genes and whole-community shotgun metagenomic sequencing were applied to investigate the shifts in soil microbial community structure and functional potentials under R. glutinosa consecutive monoculture. The results from barcoded pyrosequencing of 16S rDNA genes showed consecutive monoculture of this plant clearly affected the root-associated bacterial communities in both the rhizosphere and rhizoplane. R. glutinosa monoculture significantly decreased the relative abundances of the phyla Firmicutes and Actinobacteria and the families Pseudomonadaceae (i.e. Pseudomonas), Bacillaceae and Micrococcaceae, while increased the relative abundances of the families Sphingomonadaceae and Xanthomonadaceae. Metagenomics analysis also showed R. glutinosa monoculture significantly decreased the relative abundances of Pseudomonadaceae and Pseudomonas while increased the relative abundance of Sphingomonadaceae. Moreover, dual culture demonstrated the decline in the abundance of *Pseudomonas* spp. with antagonistic activity against fungal pathogens under monoculture. Besides, comparative metagenomics analysis showed that for potentially harmful/indicator microorganisms, the percentages of reads categorized to defense mechanisms (i.e. ABC transporters, efflux transporter, antibiotic resistance) and biological metabolism (i.e. lipid transport and metabolism, secondary metabolites biosynthesis, transport and catabolism, nucleotide transport and metabolism, transcription) were significantly higher in the two-year monocultured soil than in the newly planted soil, but the opposite was true for potentially beneficial microorganisms, which might disrupt the equilibrium between beneficial and harmful microbes. In conclusion, our results provide important insights into the shifts in genomic diversity and functional potentials of rhizosphere microbiome in response to *R. glutinosa* consecutive monoculture.

Keywords: *Rehmannia glutinosa*, replant disease, barcoded pyrosequencing, metagenomics, soil microbiome, functional potential

### Analysis of fungal community structure variation on successive rotations of *Casuarina equisetifolia* as determined by pyrosequencing

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The reproduction of *Casuarina equisetifolia* are severely compromised under the successive monoculture regime. In this study, the aim was to analyse the shifts of rhizospheric microbial community structure under the increasing years of successive rotations conditions by applying the technology of deep pyrosequencing. The results demonstrated that there were obvious differences among the first rotation *Casuarina equisetifolia* plantation (FCP), the second rotation plantation (SCP) and the third rotation plantation (TCP). In FCP, the fungal community showed a significantly higher both the Shannon and Simpson diversity indexes than in SCP (P<0.05). The relative abundance *Fusarium*, *Thelephora*, *Hortaea* and *Penicillium* significantly increased under the successive rotations. Oppositely, the *Tolypocladium and Trichoderma*, conducted a significantly decrease. The results revealed that long-term successive rotations of *C. equisetifolia* induced the alternations in the rhizospheric soil microbial community structure.

Key words: *Casuarina equisetifolia*, successive rotation, microbial community composition, deep pyrosequencing

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### Allelopathic Water Extracts Helps Improve Drought Resistance during Reproductive Stages in Wheat

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This experiment was conducted in pots where two wheat cultivars viz., Mairaj-2008 (M-2008) and Faisalabad-2008 (FD-2008) were grown in the pots filled with 10 kg of the soil. The pots were irrigated as per need of the crop until leaf boot stage at 75% WHC which was followed by imposition of drought stress at reproductive (booting and anthesis) and grain filling (dough stage) stages in both wheat cultivars. When one week passed away after foliage application of water extracts on sorghum, brassica, moringa and sunflower, the drought was imposed at each stage by maintaining the pots at 35% WHC. Drought stress negatively altered performance of wheat crop in terms of morphology and yield; however, the yield of wheat under drought was improved after the foliage application of allelopathic water extracts. M-2008 performed better than FD-2008 under drought stress which was visible through improvement in the performance of this cultivar under terminal drought stress. Although, application of all the AWEs improved the performance of both cultivars, the water extract of sorghum was most useful under drought stress than other extract. Drought stress imposition at booting and anthesis was more injurious to crop than when imposed at grain filling. In crux, foliage applied AWEs applied alone or in combination might be pragmatic option to improve wheat performance under climate change.

Key words: Drought, wheat, morphology, climate change, water extract

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### Influence of different allelopathic weed management strategies on wheat performance in different wheat-based cropping systems of Punjab, Pakistan

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Weed infestation is a major constraint reducing crop productivity all over the world. In various agro-ecosystems, the weeds are impacted and prevailing cropping systems and the weed control methods. Herbicides are sole method of weed control in most of the cropping systems; however, the method is facing problems such as evolution of herbicide resistance in weeds. Moreover, recent cropping systems are highly simplified which are allowing the best adapted weed species to proliferate in the field. To solve these issues, crop rotation is a viable option for preventing weed proliferation in a specified cropping system. The blending of crop rotation with weed management practices seems highly effective in this regard. Hence this two-year study was conducted to study the effect of wheat-based cropping systems viz. fallow-wheat (FW), rice-wheat (RW), cotton-wheat (CW), mungbean-wheat (MW) and sorghum-wheat (SW) on weed infestation, wheat allometry and productivity under divergent weed management strategies viz. false seedbed, allelopathic water extracts and herbicides application while weed free and weedy check were maintained as controls. Wheat growth was substantially low when planted after sorghum due to its allelopathic affects; nonetheless, mungbean favored the wheat growth. Chemical weed control decreased weeds infestation which was followed by use of allelopathic extracts; while false seedbed was least effective.

Keywords: Weed managing strategies; Crop allometry; Cropping systems; Wheat performance

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### Exogenous of moringa plant residues and aqueous extract at low and high concentrations on germination, growth and yield of sunflower

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Moringa oleifera belonging to family Moringaceae, is considered as one of the active allelopathic plant. Allelopathic interactions are considered as concentration dependent. In this study the allelopathic potential of Moringa oleifera was studied at both low and high concentration of plant material on growth and yield of sunflower. A pot trial was conducted in Completely Randomized Design with four replications at the research area of Ghazi University, Dera Ghazi Khan Punjab Pakistan. Leaves of Moringa were applied as soil mixed and surface application at the rate of 0,100,200 and 300gpot<sup>-1</sup>. Addition of leaves in the pots increased shoot fresh (39-43%) and dry weights (23-44%), root fresh (44-74%) and dry weights (23-35), head diameter (7-14%), achene's count (5-21%) and achene's yield per plant (9%), and 1000 achene weight (1%) at lower concentration @100-200gpot<sup>-1</sup>. The improvement in these parameters was higher @100gpot<sup>-1</sup> as compared to 200gpot<sub>-1</sub>. But at higher rate (a)300gpot<sup>-1</sup> these parameters were adversely affected by Moringa as compared to control (-1 to -24%). Mixing of leaves with soil perform better as compared to surface application (1-14%). It is concluded that Moringa residues have promotive effect up to a certain concentration but at higher concentration they have inhibitory effects on growth and yield of sunflower.

Keyword: *Morinaga oleifera*, Allelopathy, Sunflower, promotive effect, inhibitory effect

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### Rhizosphere responses to environmental conditions in the *Radix pseudostellariae*

### rhizosphere under continuous monoculture regimes

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*Radix pseudostellariae (Radix Pseudostellariae* L.) is one of the most common and highly demanded Chinese medicines. High-quality *Radix pseudostellariae* is mainly produced in ZheRong, Fujian province in southern China, where the soil and climate conditions are the most favorable for its growth. However, consecutively monocultured *Radix pseudostellariae* are prone to severe diseases, which may result in reduced biomass, especially of tuberous products. This phenomenon is known as replanting disease or soil sickness. More than 70 % of medicinal plants, especially tuberous root medicinal plants, are reported to be attacked by various replanting diseases. Therefore, replanting disease incidence has resulted in a tremendous hurdle to obtain maximum agricultural production of *Radix pseudostellariae*.

The root exudates of *R. pseudostellariae* can deteriorate the microbial community in the rhizosphere, including hosting specific pathogens at the expense of beneficial microorganisms. We found the effect of phenolic acids and organic acids were invoked as a driver of the changes seen in the rhizosphere soils. Then we study the effects of an artificially applied root exudates (organic acids, phenolic acids) of Radix pseudostellariae, on Radix pseudostellariae seedling growth, rhizosphere soil microbial communities, and soil physicochemical properties. The deep pyrosequencing and qRT-PCR analysis demonstrated that the treatment of phenolic acids significantly decreased the relative abundance of Penicillium, Pseudonocardiales, Xanthomonadales, Trichoderma, Streptomycetales, Micrococcales, Gemmatimonadales. And the organic acids had significantly negative effect on the relative abundance of *Pseudonocardiales* and *Streptomycetales*, which significantly increased the abundance of Fusarium, Xanthomonadales, Micrococcales and Gemmatimonadales. The non-invasive micro-test technique analysis indicated that the root exudates increased the H<sup>+</sup> efflux in the pathogenic fungi (Fusarium oxysporum and Talaromyces helicus) and decreased the H<sup>+</sup> efflux in the beneficial fungi (Trichoderma harzianum), which creates an acid environment for the proliferation inhibition of beneficial bacteria and build-up of specialized plant pathogens. This study explains the reasons why the root exudates are able to mediate a microflora shift and structure disorder in continuously monocultured Radix pseudostellariae rhizosphere soil.

Key Words: *Radix pseudostellariae*; Replanting disease; root exudates; non-invasive micro-test technique; deep pyrosequencing

### Advanced utillization of allelochemicals in Japanese cherry blossom

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Japanese cherry blossom, "Sakura", is a very famous tree in Japan because of their beautiful flowers. There are many species and varieties in its genus. We have already reported a part of their allelochemicals in leaves of a species of Japanese cherry blossom. Many pleople have been focusing on the existence of allelochemicals in "Sakura" which contribute to human health. Actually, we identified chemicals in the extract from leaves of a species that showed two enzyme's activities related to our health. During our experiments and reporting our research, the amount of general information related to "Sakura" increased in a field of food or dietary supplements. At this time, we will show some of the functional substances in the extracts from leaves of Japanese cherry blossoms and an increase in the amount of general information related to "Sakura".

Materials were collected from University of Tsukuba. The leaves were dried and stored at room temperature until starting the extraction. Functional substances were extracted with water from leaves because we need an easy method for getting the functional substances. This process is like making tea. General information related to "Sakura" was found in several newspapers in Japan.

We will show the advanced utilization of allelochemicals and discuss the future of the allelopathy study.

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4th International Conference of Asian Allelopathy Society

## **O-26**

### Allelopathic and antimicrobial study of *Acacia* modesta and *Buxux papillosa* available in District Hangu, KPK, Pakistan

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The current study was to asses' allelopathic activities and antimicrobial potential of Acacia modesta Wall and Buxus papillosa C. K. Schneider. A laboratory based studied was made to investigated the allelopathic potential of A. modesta and B. papillosa leaves and roots on Lettuce seed (Lactuca sativa L. v. Great lakes 366). Results were significant which showed that 100 g/L water extraction of leaves of both the plants having high allelopathic potential to inhibit the seedling growth of lettuce seeds. Roots water extractions of both the plants induce seedling growth of tested plant. The in vitro antimicrobial investigations of crude methanolic extracts of A. modesta (bark and root) and B. papillosa (leaves, stem and root) plants were analyzed against five pathogenic bacteria including one Gram positive Bacillus subtilis, four Gram negative i.e. Escherichia coli, Klebsiella pneumonia, Xanthomonas campestris and Agrobacterium tumefaciens using disc diffusion method. We obtained significant results from this experiment. It was observed that K. pneumonia was the most resistant bacterium to all extracts. Present study concluded that these plants are good sources of antibacterial compounds for the curing of many pathogenic diseases. These results confirm strong allelopathic and antimicrobial potential of these both of these candidate species.

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### Remediation of deterioration in microbial structure in continuous *Pinellia ternata* cropping soil by crop rotation

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*Pinellia ternata* is a traditional Chinese herb that has been shown to suffer from continuous cropping, which significantly decreases both yield and quality. The influence of continuous cropping on microbiome in P. ternata rhizosphere and effects of remediation on microbiota by rotational cropping were assessed Illumina high-throughput sequencing technology. Microbial by using composition analysis showed that microorganisms belonging to Proteobacteria, Acidobacteria, and Actinobacteria dominated in all cropping treatments. Moreover, continuous cropping tended to decrease a-diversities along with cultivation time, whereas rotational cropping tended to increase them. Differentially abundant analysis showed that microbial structure was important for maintaining the health status of P. ternata rhizosphere. Results suggested that continuous P. ternata cropping may result in simplification and shifts in microbial structures leading to rhizosphere deterioration. Meanwhile, rotational cropping between P. ternata and wheat can revert structural changes and thus remediate the rhizosphere. PCoA and hierarchical analyses generated three post hoc clusters of sampled soils, which were named as healthy, health-suppressed, and health-depressed soil, respectively. We also found that these three types of soils represented different soil physicochemical properties and microbiome activities. Based on this finding, potential indicator taxa that respond to existing soil were also identified.

Key words: microbial diversity, community structure, differentially abundant taxa, functional features, potential indicators

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# Expression difference between allelopathic and non-allelopathic rice cultivars

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Rice allelopathy means that allelochemicals from rice are secreted around the rhizosphere and suppress the growth of weeds. The process is regulated/induced by many genes such as signaling transfer from the donor plant to target plant and allelochemicals released from the donor plant. Phenolic compounds were earliest found as one of bioactive chemiclas from rice. C4H (cinnamic acid 4-hydroxylase) catalyzes a key reaction in the biosynthesis of a large number of phenolic compounds in higher plants, also is a gene coding for cinnamic acid to p-coumaric acid, which is closely related to allelopathic activity. There are 4 genes encoding C4H in rice. In this study, allelopathic and non-allelopathic rice PI312777 and Lemont were used as materials, the full length sequence of 4 C4H gene from the two rice were cloned and sequence alignment, gene expression was detected by q-PCR after treated for certain time by SA (salicylic acid), JA (jasmonic acid), UV-B and barnyardgrass, and then a target gene was selected for genetic transformation in order to further understanding the mechanism of rice allelopathy regarding phenolic compounds. The results showed that the lengths of amino acids from the two rice lines were almost same, and the similarity was about 98.69% with typical domain of P450, and a variety of cis-acting elements such as transcriptional regulatory factors existed in the promoter of the two rice. The expression of the 4 C4H genes in time and pattern was differ under stress condition, in which Os01g0820000 and the Os02g0467000 had similar expression level after treatment by JA and SA, but Os02g0467600 and Os05g0320700 were up-regulated in PI312777 much more than that in Lemont. After 1 h treatment by UV-B or 7 days by barnyardgrass, the expression of Os02g0467600 was increased in the 2 rice lines, but up-regulated for PI312777 and down-regulated for Lemont. The expression level of the genetically transformed rice with Os02g0467600 was increased at 26.41%. These results suggested that C4H-encoding gene Os02g0467600 plays an important role in rice allelopathy to weed. There are 7 difference loci in the amino acid sequence between allelopathic and non-allelic rice, and a large number of transcriptional regulatory factors in the promoter sequence which may result in difference of allelopatic potential of rice cultivars under stress condition. Further study will be continued to confirm.

Key words: rice allelopathy; cinnamic acid-4-hydroxylase; Os02g0467600; stress; level of gene expression.

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## **O-29**

### Chemical Composition and Behavioral Responses of Essential Oil Isolated from *Eupatorium adenophorum* to *Lasioderma serricorne*

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Essential oil was extracted from the fresh leaves of *Eupatorium adenophorum* through hydro-distillation, using a Clevenger type apparatus. The responses of *Lasioderma serricorne* to the essential oil were evaluated using a Y shaped olfactometer. Unsexed adult tobacco beetles were attracted to odor from the essential oil when the amounts of essential oil were in the range from 0.1  $\mu$ g to 100  $\mu$ g. When the amount of essential oil went up to 1000  $\mu$ g, the beetles were repelled to odor from the essential oil. Beetles did not respond better to odor from the essential oil when the amount of essential oil was 0.01  $\mu$ g. In this case numbers of beetles that moved upwind toward the odor were not significantly higher than numbers of that moved upwind toward blank control.

Chemical composition analysis of the essential oil was carried out on a combined gas chromatograph-mass spectrometer (GC-MS) fitted with an HP-5MS capillary column at a temperature programme of 40 to 250°C at a rate of 5°C·min-1 and held for 10 min. The chemical components of the essential oil were identified by comparing the obtained mass spectra of the analytes with those of authentic standards from the NIST 08 library with a resemblance percentage above the ninety percent. The chemical components relative content was calculated based on the peak area of total ion current chromatogram. The major components identified in the essential oil include: (-)- $\delta$ -Cadinol 18.96%,  $\beta$ -Bisabolene 7.24%,  $\alpha$ -Bisabolol 7.071%, (-)-Bornyl acetate 6.84%, Cadinene 5.78%, (Z)-  $\beta$ -Farnesene 4.66%,  $\beta$ -Cubebene 4.64%, Velleral 4.54%,  $\beta$ -Caryophyllen 4.21%.

Key words: behavioral responses, *Lasioderma serricorne*, essential oil, *Eupatorium adenophorum*, chemical components

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## **O-30**

# Plant-soil negative feedback in a sanqi production system mediated by root exudates and soil microbe

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Replant failure caused by negative plant-soil feedback (NPFS) in agricultural ecosystems is a critical factor restricting the development of sustainable agriculture. Sanqi ginseng [Panax notoginseng (Burk.) F. H. Chen], a member of the Araliaceae family, isone of the most important herbal medicines in China and is in high demand. In China, the commercial cultivation of Sangi ginseng has been practiced for more than 200 years. Because ginsenosides have significant effects against cancer and cardiovascular disease, requirement for these compounds have increased rapidly. Recently, more than 4 million hectares of Sanqi ginseng have been sown annually in China. However, Sanqi ginseng plants are often hampered by replant failure, which results in yield reductions and other difficulties when re-establishing plants in cultivated fields due to low seed germination, poor seedling growth, and severe disease. Many factors potentially contribute to this problem, including the deterioration of soil physicochemical characteristics, nutrient imbalance, soil-borne diseases, and autotoxicity. In the last three decades, researchers and farmers have hypothesized that Sanqi ginseng replant failure results from the accumulation of pathogens in the soil. Many types of soil-borne pathogens that cause diseases in Sanqi ginseng have been identified, including fungi, Oomycetes, bacteria, nematodes, and viruses. However, Sanqi ginseng cannot be successfully cultivated again on the same land even after sterilizing the soil by using fungicides or fumigation. In addition, many tested fertilizer application and soil modification practices have yielded unsatisfactory results. Recently, it was hypothesized that the secondary metabolites released by plant residues and root exudates that accumulated in the soil could be autotoxic to P. notoginseng during replanting. Autotoxicity, which is defined as a deleterious allelopathic effect among individuals of thes ame species, has been documented in various crops. For many medicinal plants, suchas Rehmanni aglutinosa, Pseudostellaria heterophylla and Salvia miltiorrhiza, autotoxicity results in significantly reduced yields and quality. The autotoxicities in ginseng (P. ginseng) and American ginseng (P. quinquefolius), which belong to the same genus as Sanqi ginseng (P. notoginseng), were recently reported as one possible factor that contributes to replant failure. Some phenolic acids in the root exudates and rhizosphere soils of American ginseng have been identified as potential autotoxic compounds. However, phenolic acids have frequently been identified in other crops as putative

allelopathic substances and have been identified in uncultured soil. Thus, we speculated that other special autotoxic compounds might result in Sanqi ginseng replant failure. Ginsenosides are the primary biologically active compounds that are produced by Sanqi ginseng plants. At least 20 different ginsenosides occur in Sanqi ginseng, which account for more than 6% of the plant's dry biomass. Ginsenosides can be released into the rhizosphere soilas root exudates, by leaching and volatilization, or from the decomposition of plant residues by Sanqi and American ginseng. The root exudates, root extracts, and extracts from consecutively cultivated soils showed significant autotoxicity against seedling emergence and growth. Ginsenosides, including R1, Rg1, Re, Rb1, Rb3, Rg2, and Rd, were identified in the root exudates. Furthermore, the ginsenosides, R1, Rg1, Re, Rg2, and Rd, caused autotoxicity against seedling emergence and growth and root cellvigor at a concentration of 1.0 µg/mL.

We elucidated the response of *P. notoginseng* to the autotoxic ginsenoside Rg1via transcriptomic and cellular approaches. Cellular analyses demonstrated that Rg1 inhibited root growth by disrupting the cell membrane and wall. Transcriptomic analyses confirmed that genes related to the cell membrane, cell wall decomposition and reactive oxygen species (ROS) metabolism were up-regulated by Rg1 stress. Further cellular analyses revealed that Rg1 induced ROS ( $O_2^-$  and  $H_2O_2$ ) accumulation in root cells by suppressing ascorbate peroxidase (APX) and the activities of enzymes involved in the ascorbate-glutathione (ASC-GSH) cycle. Exogenous antioxidants (ASC and gentiobiose) helped cells scavenge over-accumulated ROS by promoting superoxide dismutase (SOD) activity and the ASC-GSH cycle. Collectively, the autotoxin Rg1 caused root celldeath by inducing the over-accumulation of ROS, and the use of exogenous antioxidants could represent a strategy for overcoming autotoxicity.

Ginsenosides are primary components of ginseng and play a multi-purpose ecological role. Like other saponins, ginsenosides are fungitoxic in plants and act as a host for chemical defenses at high concentrations. In the rhizosphere, ginsenosides not only exert autotoxic effects on Sanqi ginseng plants, resulting in poor defense and growth, but also stimulate the growth of soilborne pathogens. Previous reports have shown that ginsenosides can stimulate the growth of soil-borne *P. quinquefolium* and *P. notoginseng* pathogens, such as *Cylindrocarpon destructans, Fusarium solani, Phytophthora cactorum,* and *Pythium irregulare.* If the autotoxicity of ginsenosides and the infection of soil-borne pathogens act synergistically in the rhizosphere, a potentially stronger replant failure effect is expected. Some previous studies have indicated that the interactions between *Fusarium oxysporum* and autotoxic cinnamic acid may enhance soil sickness. Thus, additional studies are needed to evaluate the interactions between autotoxicity and the other factors involved in replant failure, such as soil-borne pathogens, the deterioration of soil physicochemical properties, and the imbalance of the soil microbial community. These studies could be used to understand the mechanisms of replant failure in Sanqi ginseng.

# A comparison of extraction methods of phenolic acids in cropland soil

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Phenolic acids play important roles in soil ecosystem. It is critical to measure their level in soil and various methods to extract them were used by researchers. To compare and evaluate these methods, different solvents included water, dichloromethane, 1 M and 2 M sodium hydroxide solution (NaOH) were chosen to extract phenolic acids in wheat (Triticum aestivum L.) and American ginseng (Panax quinquefolium L.) cultivated soil. All sample solutions, with or without concentrating with process, were tested high performance liquid chromatography. Nine individual phenolic acids (p-hydroxybenzoic acid, vanillic acid, syringic acid, vanillin, p-coumaric acid, ferulic acid, benzoic acid, salicylic acid and cinnamic acid) were detected in 1 M and 2 M NaOH extracts with concentrating, while only five individual phenolic acids were detected without concentrating. However, the levels of p-hydroxybenzoic acid, vanillin and p-coumaric acid detected after concentrating were significantly lower. Only five (American ginseng soil) or four (wheat soil) individual phenolic acids were detected in water extracts, and none was detected in dichloromethane extracts. The results indicate NaOH and water recovered different phenolic acids from soil, and concentrating might cause certain phenolic acids loss. Besides, concentrating may make more individual phenolic acids detectable in NaOH extract.

### Allelopathic potential of Root Endophytic fungal metabolites of *Casuarina equisetifolia*

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To determine whether endogenous fungi of Casuarina equisetifolia are involved in allelopathy, their diversity and the composition of their metabolites was determined. By Illumina Miseq high-throughput sequencing platform, a total of 318,972 sequences were obtained from 9 root samples of C. equisetifolia of different ages, which included 11 phyla, 32 families and 32 genera. Four fungi were isolated from the roots by the traditional method and identified by biolog microbial automatic analysis system as Aspergillus aculeatus lzuka, Penicillium melinii thom, Neosartoryafisccheri (Wehmer) Malloch & Cain BGA, and Penicillium solitum westling BGB. By GC-MS analysis of the fermentation broth of four endogenous fungi, the pyrogallol, lauric acid, palmitic acid, stearic acid, 2,4-di-tert-butylphenol, 1,2,3,4-butanetriol and globulol and other organic compounds were identified. Among them, 2, 4-ditert-butyl phenol, 1,2,3, 4butanediol and globulol were also found in soil, root and litters extract, through the comparing with previous GC-MS analysis of soil, root and litters extract. The results implied that root-endogenous fungi are involved in the synthesis of sensitized substances. The bioassay showed the fermentation broth of the four inhibited significantly root-endophytic fungi *Thespesia* lampas and Calophyllum inophyllum seed germination, A. aculeatus showed the strongest inhibitory effect on seed germination.

### The role of low molecular weight organic acid accumulation on microbial deterioration in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes

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A two-year field trial of consecutive monoculture of R. pseudostellariae was conducted to further understand the underlying mechanism why continuous monoculture regimes significantly decline the biomass and quality of Radix pseudostellariae. Quantitative PCR was used to analyse the total bacteria and fungi in the monocultured rhizosphere soil and the expression of the biocontrol-related genes in bacteria. And HPLC analysis was used to detect the organic acids and toxins.

HPLC analysis indicated that continuous monoculture soil was rich in small molecular weight organic acids which had cumulative effects over time in the rhizosphere soil under monoculture regimes. The qPCR analysis demonstrated the accumulated organic acids concerned in rhizosphere soil led to the higher ratios of fungi/bacteria over time. Further analysis suggested that the mixture of organic acids at the ratio and dosage close to actual situation detected in the rhizosphere soil could significantly promote the growth of the pathogenic fungi, such as Talaromyces helices, Fusarium oxysporum and Fusarium moniliforme concerned, and the chemotactic response, the expression of chemotaxis-related gene (cheA) and biofilm formation of the specific pathogenic bacterium, Kosakonia sacchari, revise is true in the case of Bacillus megaterium and Bacillus pumilus concerned. At the same time, the present result also found that the mixed organic acids stimulate the production of toxins (3A-DON and 15A-DON) as well as H2O2 generations in the specific pathogenic fungi. Furthermore, the organic acids had negative effects on the expression of the biocontrol-related genes, and hence resulted in attenuated antagonistic activities of the PGPR to suppress the mycelial growth of the pathogenic fungi at the dosage close to actual situation in the monoculture rhizosphere soil. This result unveils the mechanisms how the accumulated organic acids differentially mediate deterioration of soil microbial composition and structure in monocropping system.

4th International Conference of Asian Allelopathy Society

### **P-4**

#### Hijacking underground common mycorrhizal networks for interplant defense communication

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Majority of terrestrial plants form mycorrhizal symbiosis, which plays a vital role in plant nutrition and stress resistance. Common mycorrhizal networks (CMNs) link multiple plants together in ecosystems. Our study shows that CMNs mediate plant-plant communication between healthy plants and enemy-challenged tomato plants. After establishment of CMNs with the arbuscular mycorrhizal fungus Glomus mosseae between tomato plants, inoculation of 'donor' plants with pathogen or herbivore insect led to increases in resistance and activities of the putative defensive enzymes, as well as induction of defense-related genes in healthy neighboring 'receiver' plants, suggesting that CMNs may function as a plant-plant underground talking conduit for systemic defense. However, use of a JA biosynthesis defective mutant spr2 as 'donor' plants resulted in no induction of defense responses and no change in insect resistance in 'receiver' plants, suggesting that JA signaling is required for CMNs-mediated interplant communication. Our results indicate that plants are able to hijack CMNs for herbivore-induced defence signal transfer and interplant defense communication.

Key words: Common mycorrhizal networks; Plant communication; Induced defense

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### Changes in Amonia oxidizing community during consecutively monocultured Pseudostalleria heterophylla

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Monoculture problems occur and prevail in many crops. Psedustaleria heterophylla is one of the important medicinal crop in China, which is effected by the monoculture diseases and is becoming a daunting task to comprehend. Previous research have pointed out that soil physico-chemical (such as production of more phenolic acids) and microbial imbalance could lead to this problem. Here we explored the population abundance and diversity of ammonia oxidizing community consisting of ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB). We also quantified the other nitrogen cycle genes in the rhizosphere soil. The gene abundance of nar, nifH, nirK and nosZ were significantly lower in all treatments than the newly planted plants. The DGGE and qPCR was employed to analyze the community structure and abundance of AOA and AOB. Both molecular techniques showed that amoA gene from AOA and AOB sharply decreased in 2 year and 3 year monoculture rhizosphere soils. In contrast, the application of the bio fertilizer improved the soil rhizosphere conditions and triggered the process of reshaping the Ammonia oxidizers in the soil close to the newly planted rhizosphere soil. However, it was more in favor of AOB as compared to AOA. We concluded that using bio oraganic fertilizer could reduce the monoculture disease problems by partially reverting the ammonia oxidizing population close to newly planted ammonia oxidizers population.

#### Identification of a putative chemical floral filter in Sisyrinchium rosulatum

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Most angiosperms attract insect pollinators. Entomophily influences floral shape and pollination syndrome—floral traits that have evolved in response to different pollination needs and type of pollinators. For example, flowers visited by hoverflies are flat and have a simple shape. In contrast, flowers visited by bees have a complex shape and are deep. Flowers of annual blue-eyed grass (Sisyrinchium rosulatum) have a flat and simple shape, characteristic of the entomophily by hoverflies. However, in our study using the quadrat method, we could not observe any S. rosulatum flower-visiting hoverflies or bees during field studies conducted in 2017 and 2018. Furthermore, we could not observe any flower-visiting hoverflies (Syrphinae) for S. rosulatum in the test experiments performed in a breeding box, even though the hoverflies frequently visited another flower, wood sorrel (Oxalis corniculata). Based on the results of the preference tests conducted using a T-shaped pipe, we found that Syrphinae significantly avoided feeding on S. rosulatum (p=0.0004) and instead chose O. corniculata (p=0.0081). Recently, it has been suggested that flowers emit specific chemicals not only to attract select pollinators but also to repel other inconsequential insects. Although studies on floral scents and their repellent actions have been conducted mostly in sexually deceptive orchids and corpse flowers, our results on grasses indicate that S. rosulatum, a common weed, repels hoverflies through chemicals. Alternatively, our data on the genetics of flower color revealed a recessive phenotype in the field. The presence of interspecific hybrids between S. rosulatum and the blue-eyed grass (S. iridifolium) further indicates outcrossing, and that self-pollination does not occur in S. rosulatum. The pollinators, specifically of S. rosulatum, are yet to be clarified.

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### Screening of weed extracts for allelopathic activity on nodulation in legumes

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It is well known that allelopathy of weeds not only influence the germination and growth of other plants but also affect soil microorganisms. Allepathic activities of some weeds are known to influence the action of rhizobia coexisting with leguminous plants. Some substances having negative allelopathic activity on root nodule bacteria may possibly be used for the suppression of harmful legume weeds, such as Kudzu (Pueraria montana). Contrarily, substances having positive allelopathic activity on root nodule bacteria are expected to enhance the productivity of crops, such as soybean. Therefore, we screened the weed extracts that affect the formation of legume root nodule using soybean, which is the main crop, and *Lotus japonicas*, which is a model plant, as the test subjects. After investigating 21 weed extracts, the extracts of red clover (Trifolium pratense) and broomsedge bluestem (Andropogon virginicus) were found to suppress the development of Lotus japonicas root nodules. However, it was not clear whether Andropogon virginicus root extracts promoted, rather than inhibited, the formation of soybean root nodules.

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### Evaluation of allelopathic activities of 56 aquatic plants on seed germination and seedling growth of upland weeds

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Allelopathy is an important factor affecting the establishment of native plant species. Studies on allelopathic activity have mainly focused on the interactions of plants occurring in the same habitat area, such as on the effects of aquatic plants on aquatic weeds and the effects of terrestrial plants on upland weeds. It is believed that plants growing in the same environment develop resistance against the allelopathic substances of each other. In contrast, it is suggested that plants growing in different environments (such as in aquatic and upland areas) may not develop resistance to allelochemicals produced by each other, which may heighten the inhibitory effects upon encounter. Therefore, we evaluated the allelopathic activities of the extracts of 56 aquatic plants and the water in which the aquatic plants were cultured, on seed germination and seedling growth of upland weeds. All tested aquatic plants displayed inhibitory activities. The extracts of aquatic plants tended to strongly inhibit the elongation of the roots of upland weeds and the culture water tended to strongly inhibit the germination of upland weeds. Germination of lettuce, used as a control, was remarkably inhibited in the culture water of Daphnia lucifera, water wisteria (Hygrophila difformis), pearl grass (Micranthemum micranthemoides), and Mayaca *fluviatilis*. Germination of white clover, a leguminous weed, was remarkably inhibited by the extracts of Hygrophila polysperma. Similarly, Lolium *multiflorum*, a Poaceae weed, was remarkably inhibited by the extracts of Eleocharis vivipara. Moreover, the extracts of red Ludwigia (Ludwigia repens), Hygrophila polysperma, some Commelinaceae species from Pantanal (Brazil), and water chestnut (Trapa natans) remarkably inhibited root length elongation in both white clover and Lolium multiflorum.

### Allelopathic effect of pruned branches of kiwifruit trees on weeds

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Large amounts of residue are discharged from agricultural production and processing. In this study, the growth inhibitory activity of residual fruits and vegetable extracts, such as from orange, grapefruit, apple, kiwifruit, pineapple, tomato, radish, Chinese cabbage, cabbage, green onion, lettuce, spinach, parsley, onion, bean sprouts, carrot, burdock, eggplant, cucumber, bitter gourd, green peppers, perilla, asparagus, garlic, ginger, okra, taro, yam, potato, sweet potato, and wasabi, was evaluated. Results indicate a strong growth inhibitory activity particularly of kiwifruits. Furthermore, this activity was not only limited to the kiwifruit but also the stems and leaves of kiwifruit plants. This prompted us to focus on pruning the branches of kiwifruit, which presents as a major residue problem. So we evaluated the weed control effect of pruned branch tips of kiwifruit in the field. In the field experiments, we validated the suppression of weeds by placing the tips of pruned kiwifruit branches in the field. Moreover, we suppose that the weed control effect is not only due to the covering effect but also due to the effect of allelopathy. This is because the weed suppression effect increased by puncturing the chip treatment compared with covering the chip treatment. Currently, we are focused on identifying the allelochemicals in pruned kiwifruit branches and developing weed control material by pelletizing pruned branches.

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# Screening of weed extracts for induction of systemic acquired resistance to anthracnose.

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Recently, systemic acquired resistance (SAR) against microbial pathogens—the introduction of broad-spectrum and long-lasting immunity in non-infected tissues via induction of the plant's own defense mechanism-has gained considerable attention in crop production. While several reports have documented the antifungal and insecticidal activities of natural products, studies on the natural product-induced SAR itself are limited. In the present study, we screened leaf extracts from 203 weed species for ability to induce SAR by performing a bioassay using cucumber plants and the pathogen *Colletotrichum* lagenarium. Many of the leaf extracts examined showed antifungal properties against anthracnose when used to treat the leaves of cucumber. The weed extracts of 48 species showed an inhibition rate of 80%. Furthermore, the extracts of several weeds inhibited the anthracnose infection in the cucumber leaves even in the untreated upper leaves. For example, a high inhibition rate of >60% was obtained in the upper leaves with the extracts from the following 11 weed species: Boehmeria nipononivea, Persicaria scabra, Dianthus armeria, Ranunculus japonicus, Ranunculus sceleratus, Sedum oryzifolium, Sedum sarmentosum, Trifolium pratense, Pueraria lobata, Cuscuta pentagona, and Gnaphalium purpureum. Such inhibition of the anthracnose infection on the upper leaves of the plants could have two possible explanations. First, the antifungal ingredient in the extracts could have been transported from the lower to the upper leaves, in which case the active ingredient of the weed extracts could be a low molecular weight compound. This could be a potential precursor for the development of a new fungicide. Second, the weed extracts inoculated in the cucumber plants could have led to SAR induction. In order to confirm the latter hypothesis, we have been determining the expression of the SAR marker gene in the model plant Arabidopsis thaliana.

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### Synthesis of Molecular Probes of *Cis*-Cinnamic Acid Analog with Antigravitropic Activity

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*Cis*-cinnamic acid (*cis*-CA) was reported by Hiradate and Fujii to be a biologically active substance derived from an allelochmical produced by *Spiraea thunbergii Sieb. Cis*-CA shows significant inhibitory activity



toward plant growth.<sup>1,2</sup> Through structure-activity relationship (SAR) study about this molecule, we found that analog **1** has strong antigravitropic activity toward lettuce roots.<sup>3</sup> In this study, we investigated design and synthesis of molecular probes of **1** in order to reveal its biological mechanism.

Design of molecular probes **5** is based on our early SAR study about **1**. Bromide **2** and boronic acid **3** were used as starting materials, and sequential transformations including Suzuki coupling, HWE reaction and Mitsunobu reaction provided primary amines **4**. Finally, fluorescent groups were connected to the amino group. Totally, 12 fluorescent probes **5** were prepared. Preliminary fluorescent imaging experiments using lettuce roots indicated that the root cap was especially stained. Synthesis of photo-affinity probes will also be shown.



1) S. Hiradate, Y. Fujii, at al., *J. Chem. Ecol.* **2005**, *31*, 591. 2) S. Hiradate, Y. Fujii, *Phytochemistry* **2004**, *65*, 731. 3) manuscript in preparation.

# Characterization of growth-promoting activity of volatile substances released from *Crocus sativus*

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Plant growth-promoting activities of volatile compounds from 13 plant species were examined using lettuce seedlings (Lactuca sativa L.cv. Legacy) at the germination stage by a dishpack method to find new natural compounds that could be sources of lead compouds for environmentally safer novel plant growth regulators, and the characterization of the promising compound was examined to understand the mechanism for the promoting activity. The volatile compounds from Crocus sativus L. showed the strongest promotive effect on lettuce root growth, followed by those from Zanthoxylum piperitum (L.) DC., Mentha spicata L. and Foeniculum vulgare Mill. Gas chromatography-mass spectrometry analysis identified seven volatile compounds including safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), isohorone (3.5.5 trimethyl-2-cyclohexene-1-one) and limonene (1-methyl-4-(1-methylethenyl)cyclohexene) from C. sativus, three volatile compounds, sylvestrene (1-methyl-5-(1-methylethenyl)-cyclohexene), neryl acetate ([(2Z)-3,7-dimethylocta-2,6dienyl] acetate) and beta-myrcene (7-methyl-3-methylideneocta-1,6-diene) from Z. piperitum and eight volatile compounds including limonene, L-fencone (1,3,3-trimethylbicyclo[2.2.1]heptan-2-one) and anethol (1-methoxy-4-[(1E)prop-1-en-1-yl]benzene) from F. vulgare. Among these volatile compounds, safranal showed the highest growth-promoting activity on lettuce roots, followed by anethol, limonene and beta-myrcene. Safranal (19 µmol/L) stimulated lettuce root length up to 162% of control 3 days after treatment. In maize (Zea mays L. cv. Honey Bantam), safranal also tended to promote primary root elongation and lateral root formation at 0.9 µmol/L 3 days after treatment.

### In Vitro Bioassay of Allelopathy in Caffeine-containing Coffee Cells using Protoplast Co-Culture Method with Digital Image Analysis

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Caffeine (1,3,7-trimethylxanthine), a purine alkaloid, is known as an allelochemical in such as coffee and tea plants. To unveil the cellular mechanism(s) of allelopathy, the protoplast co-culture method for assay of allelopathy was developed in a 50 µL liquid medium using a 96 well culture plate. Inhibitory effects of caffeine and its metabolites on the growth of recipient lettuce protoplasts were reported using this method. In the present study, a unique embryogenic cell (EC) line of Coffea canephora (robusta coffee) which accumulates caffeine during subcultures was investigated. Protoplasts were isolated from four-week cultured EC using Cellulase R10 in 0.6 M mannitol, which condition was selected from 24 combinations of six kinds of cell wall degrading enzymes. Numbers of non-spherically enlarged or divided protoplasts in a Murashige and Skoog's medium containing 2,4dichlorophenoxyacetic acid and benzyladenine, sucrose and mannitol, were counted under an inverted microscope. Protoplasts densities varied from 6 x 10<sup>3</sup> to 5 x 10<sup>5</sup> / mL. The growth of recipient lettuce protoplasts were described as % of control. Coffee protoplasts stimulated the cell wall formation of recipient lettuce protoplasts, but strongly inhibited cell divisions depending protoplast densities of coffee. Furthermore, effects of coffee protoplasts on yellow color accumulation of lettuce protoplasts were quantitated by the Image J analysis of a scanned digital image of a 96-well culture plate (DIA-PP method). Differences and causes of the allelopathic activities were discussed comparing with other plant species.

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# Isolation and identification of allelopathic substances from *Lippia canescens*

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Recently, the cultivation of ground-cover plants received much attention as a new method of weed management. Because using ground-cover plants for weed management is a weed control method that does not use herbicides, it can markedly contribute to organic and environmental preservation agriculture. These plants can deprive weeds of sunlight and grow into dominant spices. Some of these plants have a competitive advantage not only because they cover weeds, but also because they exhibit an allelopathic potential. The objective of this study was to evaluate the allelochemicals produced by ground-cover plants.

To assess the allelopathic activity, the sandwich method was used. *Lippia canescens*, which exhibited significant plant growth-inhibitory activity, was selected as a plant material. The candidate allelochemicals were purified from an acetone extract by bioassay-guided column chromatography and HPLC, based on the inhibitory activity against the growth of monocot (lettuce) and dicot (timothy) seedlings. The growth-inhibitory compounds were identified by ESI-MS and <sup>1</sup>H and <sup>13</sup>C NMR spectra analysis. From *L. canescens* extract, cornoside, arenarioside and acteoside were isolated as potential allelochemicals. Whether these compounds are present at sufficient levels to exhibit an allelopathic potential is also discussed.

This study may contribute to the further utilization of ground-cover plants.

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### Assessment of allelopathic potential of some crop residues on wheat and some associated weeds (chard and wild oat)

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In Egypt a huge amount of crop residues remained in fields and are usually burnt by farmers. In this study we attempted to utilize some agriculture wastes such as rice straw, sorghum, sunflower and wild mint under both laboratory and greenhouse conditions to determine their allelopathic potential. In laboratory bioassay, sandwich method with lettuce seed has been used. The results showed that all allelopathic plants significantly reduced lettuce radical length. The highest inhibitory effect at 50 mg was observed by sunflower (82%) followed by sorghum (73%), rice straw (56%) and finally by wild mint (57%). Greenhouse experiment was carried out to evaluate the potency of using these crop residues in combating weeds under environmental conditions. Test plant powders were used at two levels 0.5 and 1.5 % as soil incorporation against two of its obnoxious weeds, chard and wild oat, and wheat. Germination percentage, chlorophyll content, root and shoot elongation, fresh and dry weight were evaluated after 30 days from sowing. Regarding sunflower residues, it achived the highest inhibitory activity against wheat and its weeds. Sunflower residues significantly inhibited germination process by (90%, 40% and 56%) and reduced root growth by (96.7%, 81.1 and 81%) in chard, wild oat and wheat, respectively comparing to control at 1.5% powder. Sorghum residues caused also high inhibitory activity against the test weeds while, its inhibitory effect against wheat was not significant. As for rice straw and wild mint, both caused subsential suppression in root growth of chard (93.8% and 97.1%, respectively). It was concluded that it is possible to incorporate the tested crop residue with soil to control weeds instead of herbicides under field conditions.

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#### Investigation of Allelochemicals Activity in Cassava Leaf and Callus for Genetic Transformation

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Cassava (Manihot esculenta) is a perennial woody shrub in Euphorbiaceae family that most important sources of food energy and industrial raw materials in tropical and subtropical regions. Cassava is the third most important source of calories and the sixth most important crop (Burns et al., 2010, doi:10.3390/su2113572). Cassava can produce some phenolic compounds which contributed to the antioxidant and antimicrobial activity. For example, ascorbic acid and  $\beta$ -carotene are known as an allelochemicals in cassava. We currently focus on the research to explore morphological and physiological responses of Asian (mainly Indonesian) cassava under drought stress. The present study was aimed to investigate allelochemicals activity of two typical cassava varieties from Okinawa Japan. At first, morphological and physiological characteristics of these local cassava varieties were evaluated. Allelochemicals activity was tested on seeds germination of Lactuca sativa, using sandwich method. Then, antimicrobial activity of cassava leaf and cassava callus was tested in bacteria species Escherichia coli JM109, Agrobacterium tumefaciens LBA4404 and A. tumefaciens EHA101 strains by measuring the growth inhibition zone and/or microplate reader based bio-assay. Differences and causes of the allelochemicals activities were discussed. The minimum zone of inhibitory between A. tumefaciens LBA4404 and EHA101 indicated as effective vehicle for plant transformation. Finally, a callus culture system was developed using petiole and stem explants on Murashige and Skoog medium with various concentrations of phytohormons (3  $\mu$ M 2, 4-D + 3  $\mu$ M BA, 10 and 50 µM picloram). The infection time and bacterial density could have some effects on the transformation efficiency.

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### Screening for Potential Allelopathic Activity of Medicinal Plant in Phnom Kulen National Park, Cambodia

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Recently, the term of allelopathy is wisely used to enhance sustainable agriculture, particularly for weed control. Medicinal plant, known as herbicide, contains many pharmacological activities are commonly used with many prescribed drugs; however their several functions including defense against insects, fungi, diseases and herbivorous mammals, are reported allelopathic properties which influent to surrounding plants for both positively and negatively (*Ahn K. 2017; Abbiw 1990; Rice 1984*). This present study aims to screen potential allelopathic activity of medicinal plants in Phnom Kulen National Park, where known the richness of medicinal plants and cultural values in Cambodia. To achieve this objective, 113 medicinal plant species from 57 families were screened by using sandwich method (*Fujii et al. 2004*). Lettuce seeds (*Lactuca sativa L.* Great Lakes No. 366) was observed as a test plant material in bioassay to determine the influent of root exudates.

As a result to allelopathic activity exhibited through plant leachates, the radicle elongation percentage of lettuce seeding were in range 4 to 118.8% and 3.1 to 98.8% for 10 mg and 50 mg sample treatment. We observed that radicle growth inhibition >90% occurred in 34 species, 70–90% in 32 species, 50–70% in 27 species, 30–50% in 13 species, and 4–30% in 7 species. Additionally, 32 plant species show the significant inhibition on lettuce radicle as evaluated by using standard deviation variance (SDV). We also found that *Iris Parllida* (4%) showed the strongest inhibitory activity among 113 test plants. Following by *Peliosanthes tesa* (8.2%), *Suregada multiflora* (21.3%), *Ervatamia microphylla* (22.4%), *Allophyllus serrulatus* (23.3%), *Eupatorium odoratum* (24.1%), and *Stephania rotunda* (28.7%). This is the first massive screening allelopathic activities of medicinal plants in Cambodia. This result could be used as a benchmark for future research on isolation and identification allelochemicals among plant species in Cambodia.

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#### **Evaluation of allelopathic activity of** *Hypericum oliganthum* -Toward practical use as cover plants for weed management and conservation of *Hypericum oliganthum*-

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Hypericum oliganthum is distributed to the western part of Kanto, Japan and Korea, living at paddy levees or riverbeds. This plant is designated endangered species rank IB. According to survey by the Ministry of Environment at 2007, average decreasing rate was 61%, so it's urgent to conserve this species. Although this plant is endangered species, propagation of this plat is not difficult (Yoshioka, 2015). Hypericum oliganthum is perennial, creeping plant. These characteristics indicate that there is a possibility to be useful as cover plants for weed suppression at paddy levees. In addition, its flowers are beautiful. If Hypericum oliganthum can be useful as cover plants, not only conservation of this, but also reduction of farmer's burden and beautification of paddy levees will be expected, so it will get easier to get consensus of people to conserve Hypericum oliganthum. On the other hand, allelopathy is one of factors related to weed suppression. However, allelopathic activity of Hypericum oliganthum is not reported, so allelopathic activity of Hypericum oliganthum was evaluated. Three methods: Sandwich method, plant box method and rhizosphere method were conducted. Allelopathic activity through leaching from dried leaves, stems, amd roots of Hypericum oliganthum were evaluated by sandwich method. By plant box method, allelopathic activity through root exudates was evaluated. Allelopathic activity of chemicals existing in rhizosphere soil was evaluated by rhizosphere soil method. As a result, inhibition percentage of radicle growth of lettuces seeds (Species: Legacy) were as follows. Sandwich method: 2h dried leaves (10 mg) 20.2 (%), dried leaves (50 mg) 50.6 (%), dried stems (10 mg) 6.5 (%), dried stems (50 mg) 46.7 (%), dried roots (10 mg) 6.4 (%), dried roots (50 mg) 39.5 (%), Plant box method: 66 (%), Rhizosphere method: dried rhizosphere soil (3.0 g) 41.3 (%). Dried leaf leachates and root exudates had high allelopathic activity.

Leaves of *Hypericum oliganthum* are dropped and accumulated on the surface of soil in winter, so chemicals related to weed growth inhibition may be leached and accumulated on the surface.

In addition to shading light by creeping, dried leaf leachates and root exudates from *Hypericum oliganthum* may contribute to weed suppression. So, possibilities for *Hypericum oliganthum* to be useful as coverplants became higher than before.

### Possible involvement of nitric oxide in promotion of rice seed germination under low temperature by inoculation of *Bacillus pumilus* TUAT1 strain

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In direct sowing culture of rice in cold areas, high seed germination rate in soil and vigorous growth of the seedling under low temperature are important traits. *Bacillus pumilus* TUAT1 strain is one of plant growth-promoting rhizobacteria. In rice seedings inoculated with TUAT1, production of nitric oxide (NO), which is a signal molecule in allelopathic interactions, was increased and it resulted in a promotion of crown root formation. In this study, the effects of inoculation with TUAT1 to seed germination in soil and seedling growth of rice under low temperature ( $15^{\circ}$ C) were confirmed. The relationship between increase of NO production and promotion of germination in rice seeds was also investigated.

The seeds of *japonica* rice 'Hitomebore', 'Fukuhibiki', 'TAT-26' and 'LTAT-29' were sown on granular soil and cultivated at 15°C. When the seeds treated with TUAT1 spore suspension (10<sup>7</sup> CFU/mL), higher germination rate was observed in all varieties. In 'Fukuhibiki' and 'LTAT-29', growth amount of seedlings was significantly increased. These results showed that inoculation of TUAT1 promotes seed germination and seedling growth of rice under low temperature.

When the seeds were treated together with NO scavenger (100  $\mu$ M cPTIO) and TUAT1, the promotion effect of TUAT1 on seed germination in soil was dissapeared. The NO production level in the seed of 'Hitomebore' estimated using DAF-FM DA was higher after treated with TUAT1 than control seeds. These results suggest that possible involvement of the increase in NO production level to the promotion of germination under low temperature in rice seeds treated with TUAT1.

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### Enumeration of soil microorganism which degrade L-3,4-dihydroxyphenylalanine, an allelochemical of *Mucuna prurience* var. *utilis*

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Since study dealing with allelopathy have been conducted mainly in vitro. Thus, the effect of allelopathy on soil microorganism, such as allelochemicaldegrader, remain unclear. We hypothesize that the number of allelochemicaldegrader increases when allelochemical donor plants are grown in soil. Mucuna prurience var. utilis, a tropical legume, is grown for edible bean, cover crop and green manure in south America or southeast Asia, M. prurience has been known to have allelopathic effect. Experiments in vitro suggest that L-3,4dihydroxyphenylaranin (L-DOPA) is a putative allelochemical and inhibits growth of plants. In this study we measured the number of L-DOPA-degrading microorganism in several soils by using MPN method and the amount of L-DOPA in soil in which *M. prurience* was grown. More than 10<sup>5</sup> MPN g<sup>-1</sup> of Lthere was no significant difference in the number of L-DOPA-degrading microorganisms between M. prurience grown soil and no-growth soil. The amount of L-DOPA in M. prurience grown soil was lower than detection limit (<2.48  $\mu$ g g-1). These results suggested that L-DOPA released from M. prurience may be degraded by soil microorganisms because they are at high density in soil or the amount of L-DOPA released from Mucuna prurience var. *utilis* is very small. Further studies would be needed to verify these hypotheses.

### Phytotoxic effect of soil-incorporated dried leaf residue of *Euphorbia golondrina* L.C. Wheeler on tomato growth and yield

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Invasive alien plant species play a major role in agricultural and natural ecosystems by determining vegetation pattern, plant dominance, and yield loss. Euphorbia golondrina, an annual native to Central America, is now reported in Cameroon where it is a nuisance in farming systems. This study aimed to evaluate the phytotoxic effects of E. golondrina soil-incorporated dried leaf residue on growth and yield of tomato in the Greenhouse of the University Buea, Cameroon. Incorporation of the leaf residue to the soil showed chlorosis, necrotic lesions and wilting of tomato under treatments 10 g and 17 g at 2 WAP. The number of leaves, leaf area, dry shoot and root weight of tomato grown in the soil-leaf mixtures were also significantly reduced. The inhibition percentages due to the addition of the three concentrations of E. golondrina dried leaf residue on the dry shoot weight at 4 WAP were 42.1 g, 49.6 g and 75.9 g for tomato respectively. Similarly, declines in the dry root weight ranged from 42.9 to 57.1% under amended soil treatments. Further, the dry fruit yield and shoot weight of tomato under the treatments 7 g, 10 g and 17 g reduced with increase in leaf residue concentrations. The reductions in the fruit yield and fresh head weight caused by treatments 10 g and 17 g were 54.2 and 71.4% respectively. The mineral contents in the leaves of tomato showed significant differences in the uptake of N, Mg, Na, Cu and Fe by the tomato plants. However, the P content was relatively constant in the leaves of tomato at 4 and 12 WAP. At 12 WAP, the Fe content in soils with treatments 10 g and 17 g was markedly enhanced in comparison with the other nutrients. Hence, E. golondrina is phytotoxic to tomato and warrants phytochemical analysis.

#### Endophytic Fungi Isolates from an Allelophatic Plant: The Case of *Vicia villosa* Roth

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Plant-microbes symbiotic such as mycorrhizal fungi and some endophytes play a vital role in ecosystem services and function by enhancing nutrient uptake and growth, and/ or deter antagonistic microbes attack.

Fungal endophytes are fungi that spend either full or a considerable part of their life inside living plant tissues without causing any visible harm on the host. Secondary metabolites produced by fungal endophytes are useful resources for natural products which have a wide range of applications in medicine and agriculture. Hairy vetch (*Vicia villosa* Roth.), an allelophatic legume native to some part of Europe and western Asia such as Japan contains the secondary metabolite cyanamide responsible for allelophatic potential and serve as the central ingredient in nitrogen fertilisers. Previous studies have elucidated the cover crop attributes and secondary metabolites from *V. villosa* vegetative parts but none have determined the endophytic profile. Hence, this study was aimed to document the endophytic mycoflora diversity of V. *villosa* with the view of exploitation in biotechnological applications.

Random samples, from asymptomatic surface sterilized tissues (root, stem, leaf) from *V. villosa* were used for fungal assemblage and diversity investigation following comparative morphological descriptors with the aid of a light microscope. Forty-three endophytic fungi were isolated from *V. villosa*. The isolates were characterized and grouped according to culture characteristics and colony growth morphology. Thirty-six morphotypes, equivalent to 83.7% were assigned to ascomycotina. Twenty-seven isolates, equivalent to 62.8% were found in the leaf tissues.

Among the isolated endophytic fungi from leaves, 15 and 1 isolates were confined to root and stem tissues, respectively. Further, *penicillium spp*. were the most dominant endophytic fungi in all tissues.

This study provides a base line data on the fungal association of *V. villosa* for usage as an eco-friendly source of natural metabolites in the medical and agricultural industries.

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### A Perspective on Allelopathic Potential of Some Turkish Plant Species by Sandwich Method

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Turkey is one of significant country in the world from the stand point of plant genetic resources and plant diversity. Although Turkey's flora presents potential for allelopathy researches, there is a lack of studies that screen the large number of Turkish plant species by bioassasy for allelopathic activity. That is why, the main objective of current research is to screen allelopathic activity of some crops, herbs, spices, medicinal and aromatic plants from Turkey and to determine strong allelopathic species for next studies. In the present study, 165 dry plant samples supplied from herbalists in different cities in Turkey and were assayed by the Sandwich Method for their allelopathic activity. The Sandwich Method is a bioassay method which is reliable, effective, less time-consuming, and widely used in determining allelopathic effects of dry plant materials under laboratory conditions. Lettuce seeds (*Lactuca sativa* L.) was used as a test plant material in the bioassay. Inhibitory activity of dry plant samples against lettuce seeds with that of control seeds.

Top ten plant species, which have had maximum inhibition activity on the radicle lengths of treated seeds, were *Punica granatum* L. (Lythraceae), *Alcea rosea* L. (Malvaceae), *Lavendula stoechas* L. (Lamiaceae), *Silybum marianum* (L.) Gaertn. (Asteraceae), *Nigella sativa* L. (Ranunculaceae), *Camilla sinensis* (L.) O. Kuntze. (Theaceae), *Pinus brutia* Tenore (Pinaceae), *Liquidambar orientalis* L. (Altingiaceae), *Malva sylvestris* L. (Malvaceae), and *Citrus sinensis* (L.) Osbeck. (Rutaceae).

This is the first comprehensive report on screening of over hunder number of Turkish plant species by bioassasy for their allelopathic activity. Further studies would be applied to determine allelochemicals which cause allelopathic activity in regarding plants. Presented data could be utilized as benchmark information to develop natural herbicides for weed control.

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### A perspective on allelopathic activity of some Turkish plant species by Dish Pack Method

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Although Turkey's flora with its existing diversity present potential for allelopathy researches, there is a lack of studies that screen the large number of Turkish plant species by bioassasy for allelopathic activity. That is why, the main objective of current research is to screen allelopathic activity of some crops, herbs, spices, medicinal and aromatic plants from Turkey and to determine strong allelopathic species for next studies. In the present study, 79 dry plant samples supplied from herbalists in different cities in Turkey and were assayed by the Dish Pack Method for volatile allelopathic activity. The Dish Pack Method is a quick bioassay method which is widely used in screening for the presence of volatile allelochemicals from plant materials under laboratory conditions. Lettuce seeds (*Lactuca sativa* L.) was used as a test plant material in the bioassay, because of its high sensitivity to inhibition by allelochemicals and reliability for germination. Inhibitory activity of dry plant samples against lettuce seeds with that of control seeds.

Top ten plant species, which have had maximum inhibition activity on the radicle lengths of treated seeds, were *Centaurium erythraea* Rafn (Gentianaceae), *Hibiscus rosa-sinensis* L. (Malveceae), *Salvia officinalis* L. (Lamiaceae), *Lavendula stoechas* L. (Lamiaceae), *Valeriana officinalis* L.(Caprifoliaceae), *Melissa officinalis* L. (Lamiaceae), *Prunus mahaleb* L. (Rosaceae), *Hypericum perforatum* L. (Hypericaceae), *Ceratonia siliqua* L. (Fabaceae), and *Rosmarinus officinalis* L. (Lamiaceae). This is the first comprehensive report on screening of large number of Turkish plant species by bioassasy for volatile allelopathic activity. Further studies would be applied to determine allelochemicals which cause allelopathic activity in regarding plants. Presented data could provide not only a pathway to identify new natural chemicals that would serve sustainable agriculture, but also it could be utilized as benchmark information to develop natural herbicides for weed control.

### Effect of Mechanical Weeding on the Allelopathic Potential of Weed

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The allelopathic contribution on both vegetation growth inhibition and species richness depression by trampling and cutting of an herbaceous plant community will be evaluated. In fact, plant wounding by either biotic or abiotic agents induces an emission of allelochemicals, mainly volatile, such as leaf aldehydes, leaf alcohol and ethylene, from wounded planted. Therefore, in the no-till system, the vegetation will be trampled and removed using a grass roller and a grass trimmer, respectively, as mechanical plant wounding agents. Four replications of five treatment and control (undisturbed) plots were randomly designed in the field experiment located in Tokyo University of Agriculture and Technology. The five treatments include vegetation cutting and four different trampling intensities: 25, 75, 200 and 500 Passes. Species richness will be recorded before and after treatments. Plant coverages and heights of the candidate plants species will be recorded one day before, as well as, 15; 30; and 75 days after treatment to investigate the species traits, resilience and resistance. The soil hardness tester will show the difference between soil hardness in control and treated plots. Solid Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrum (GC-MS) will identify leaf aldehydes. While ethylene will be detected by Gas Tech System. Moreover, a bio-assay will be conducted to check the effects of aldehydes and ethylene on the growth of lettuce and the candidate species in the laboratory. The rhizosphere soil method will reveal the increase of soil allelopathy, due to increased root exudation of allelochemical after plant wounding. Eventually, based on the study findings, we intend to suggest grass rolling as a new eco-friendly weeding method.

### Efect of relay living mulches with maiz (Zea mays L.) on weed communities under reduced soil-tillage

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The Shift from conventional to reduced soil-tillage lead to the development of high weed infestation pushing farmers to intensively use herbicides. However, one of the sustainable alternatives to reduce the demand for herbicides is the use of living mulches. Field experiment was conducted in the rainy season 2017/2018 at Sussundenga research station, Manica province (33°15'E 190 20'S and 635m at sea level) to investigate the effect of relay maize with brachiaria (Brachiaria decumbensis), velvet bean (Mucuna prurens), cowpea (Vigna unguiculata L.), soybean (Glycine max) and sunhemp (Crotalaria juncea) compared with sole maize on weed composition and diversity; maize yield. In addition, living mulch soil coverage (%) and height were recorded to determine multiplied dominance ratio (MDR), as well as aboveground biomass. Data were recorded at 35 and 83 days after planting (DAP) and the end of grain filling. The MDR of living mulches showed a tendency to increase markedly during the maize growth and better performance was observed under velvet bean (94.5m<sup>3</sup>m<sup>-2</sup>) and brachiaria (68.5m<sup>3</sup>m<sup>-2</sup>). From the results of analysis of weed functional groups, a significant decrease was observed in annual monocotyledonous grasses under brachiaria and perennial dicotyledonous broadleaved under cowpea. Furthermore, brachiaria, velvet bean and soybean reduced weed density by 58.5%, 46.3% and 43.6% more than under sole maize respectively. On another hand, the Leguminous living mulches showed a tendency to boost the weed aboveground biomass production opposing the weed density pattern. In addition, weed diversity among treatments did not vary during the maize growth. The introduction of a different type of living mulches had no significant effect on maize grain and stover yield. However in terms of total plot biomass produced (maize stover yield + living mulches aboveground biomass) under brachiaria there was an increase by up to 11174.4 to 13957.8 kg/ha more than sole maize and within living mulches, except under mucuna which outperformed sole maize, intercropped sunhemp, soybean and cowpea by up to 5450.8 to 8234.2 kg/ha. Thus, under reduced soil-tillage, relaying maize with brachiaria and velvet bean could be an appropriate strategy to suppress weed, reduce heavy use of herbicides and biomass production. However further studies on the effect of sowing date and densities are critical issues to improve maize productivity.

# Presence and variations of *l*-canavanine in hairy vetch (*Vicia villosa*) during seeds maturation stages

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1-canavanine is a nonprotein amino acid with allelopathic potential that has recently been found in several leguminous species. Due to its unique structure and its potential in many domains such as medicine and agriculture, 1-canavanine has drawn much attention. Recently, 1-canavanine has been recognised as precursor for cyanamide biosynthesis in young seedlings of hairy vetch (Vicia villosa). However, quantification and qualification of this compound in mature plant and during seed maturation stages has not been reported. Therefore, we assessed the allelopathic activity and quantified the l-canavanine in different parts of the mature plants of hairy vetch by using GC-MS. Sandwich method of immature seeds showed stronger inhibitory activity in comparison to the other parts of the plant. EC<sub>50</sub> of l-canavanine was estimated as 5.5 µmol/ml. We found no l-canavanine in the leaf, stem, and root of hairy vetch. However, it was found that 1-canavanine is biosynthesised in high concentration in immature seed during the globular (6µg/100mg) and increases throughout the cotyledon, early maturation and fully maturation stages in the seed (400 ng/100mg). These results were consistent with the bioassay of aqueous ethanol extract of hairy vetch immature seeds. We think that incorporation of hairy vetch into the soil during the seed maturation stage maybe increase its weed controlling potential in the field condition.

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### Contents of *l*-Canavanine and Free Amino Acids in Germinated Seeds and Protoplasts of Etiolated Seedlings of Hairy vetch (*Vicia villosa*).

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*l*-Canavanine is known as a precursor for biosynthesis of cyanamide, the main allelochemical in leaves of hairy vetch (V. villosa var. Kantaro). In the protoplast co-culture bioassay method of allelopathy, epicotyl protoplasts of etiolated seedlings and *l*-canavanine were both strongly inhibitory, while cyanamide was not so at low concentrations. In this report, we quantified the l-canavanine and free amino acids (FAAs) concentrations in tissues of germinated seeds and protoplasts of etiolated seedlings by using GC-MS. Major transport and storage compounds (FAAs), ASN and GLU rapidly decreased in epicotyl tissue after days of germination, while other FAAs increased significantly. Similarly, the *l*-canavanine content decreased, however, its concentration (20 mg/g fresh weight) at the day 7 was yet significantly higher than ASN (10 x) and GLU (7 x). *l*-Canavanine content in epicotyls protoplasts was estimated to be 0.23  $\mu$ moles /10<sup>5</sup> protoplasts and lower in root protoplasts. These concentrations agreed with our results for pure *l*-canavanine in the protoplast co-culture. Thus, in the V. villosa, l-canavanine may act as an allelopathic compound as well as transport-storage compounds similar to those of major storage FAAs. In general, it is possible that the V. villosa at early growth stage utilizes *l*-canavanine as its main allelopathic compound to combat neighboring species.

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### Evaluation of allelopathic ground cover plants and isolation of azetidine 2-carboxylic acid as allelochemical from big blue lilyturf

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For the selection of effective weed control, seven ground cover plants, creeping thyme (*Thymus serphyllum*), moss phlox (*Phlox subulata*), carpetweed (*Phyla canescens*), trailing ice plant (*Mesembryanthemum spectabile*), mondo grass (*Ophiopogon japonicus*), pennyroyal (*Mentha pulegium*), big blue lilyturf (*Liriope platyphylla*) were compared by field experiment for four years, and allelopathic activity of each plant was examined. In field experiment, dry weight of weed, cover degree, MDR, weed inhibitory rate and species of weeds were examined. Dry weights of weed at creeping thyme, carpetweed, mondo grass and big blue lilyturf were lower. Weed inhibitory rate and cover degree of big blue lilyturf was the highest. The number of emerged weed species of big blue lilyturf was the least. Allelopathic activity was evaluated by sandwich method and plant box method. Mondo grass and big blue lilyturf inhibited the growth of lettuce about 90 % by sandwich method.

Big blue lilyturf is a Japanese traditional perennial ground cover plant and showed best results both in field experiment and allelopathic activity. Allelochemicals of big blue lilyturf were evaluated by total activity. It was found that azetidine 2-carboxylic acid (AZC) is considered to be a main allelochemical of big blue lilyturf.

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### Nitrogen mineralization and microbial biomass dynamics in different soils following amendment with contrasting plant resources

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The need to evaluate common underutilized organic residues (OR) as soil amendments in small-holder agro-ecosystems is necessary. This study was conducted to evaluate the nitrogen (N) mineralization, soil pH and microbial biomass dynamics of contrasting ORs in different soils. Six ORs (Leucaena leucocephala, Centrosema pubescens, Gliricidia sepium, Pueraria phaseoloides, Azadirachta indica, and Theobroma cacao), each at 24 mg g<sup>-1</sup> dry soil was added to three soils and incubated for 120 days. Inorganic N contents varied among the ORs, soil types and incubation days. Regardless of soil type, Gliricidia had the highest net inorganic N while the lowest was in Theobroma. Nitrogen mineralization rate of 1.4 to 1.5 mg N kg<sup>-1</sup> soil day<sup>-1</sup> was respectively observed for Legon and Techiman soils, and was twice higher compared to Nyankpala. However, Nyankpala soil released higher cumulative mineralized N than Techiman and Legon, implying high N mineralization efficiency in the former. Consistent soil pH and microbial biomass C (MBC) increase were respectively observed for Theobroma and Pueraria treatments in all soils. Inherent soil properties accounted for shifts in MBC and soil organic C while OR quality indices controlled pH and N mineralization. Thus, we conclude that soil properties and OR type are important for optimal utilization of organic resources.

#### Insect antifeedant activities of sesquiterpene lactones from Asteraceae glandular trichomes

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Yacon (Smallanthus sonchifolius (Poepp. & Endl.) H. Rob) and Helianthus strumosus L. are perennial Asteraceae, and their roots are foodstuff. Since both plant species have a high tolerance to pests, they can cultivate with minimal use of pesticides. They possess a large number of glandular trichomes on leaves, which are likely implicated in resistance to herbivores. Thus, the leaves were rinsed using dichloromethane (DCM) to obtain the rinsate, and plant residues were subsequently extracted by DCM to obtain the DCM extract and these two fractions were tested on the common cutworms (Spodoptera litura F.). It was found that the rinsate showed a higher insect antifeedant activity against the common cutworms than for DCM extract. The active principle of the glandular trichomes were isolated by silica gel flash chromatography using a hexane and ethyl acetate solvent system, and were identified as the sesquiterpene lactone, (5-Acetoxy-4-epoxyangeloyloxy-6-methylcarboxygermacrenolide) Uvedalin and its oxidative congener, Enhydrin (5-Acetoxy-10-epoxy-4-epoxyangeloyloxy-6-methylcarboxy germacrenolide), from yacon, and Erioflorin, from H. strumosu. Both of Uvedalin and Enhydrin showed strong insect antifeedant activities with ED<sub>50</sub> values of 18 and 9.0 nmol/cm<sup>2</sup>, respectively, whereas Erioflorin had a moderate activity with  $ED_{50}$  value of 7.32  $\mu$ mol/cm<sup>2</sup> against the common cutworms. Thus, it is suggested that the substituents of these germacrenolide contribute, at least in part, to the insect antifeedant activity and that Uvedalin and Enhydrin serve as chemical defense weapons in yacon toward phytophagous insects.

# Weed control using herbal medicine extraction residue as natural mulch

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Although herbal medicine extraction residues are organic wastes, some of them are used to make compost for agricultural use. Therefore, we have evaluated the effects of them made from the cinnamon bark, ginger and magnolia bark on weed control in the field as well as identified components responsible for the weed control.

The field test on weed control using the residue resulting from extraction of the cinnamon (*Cinnamomum cassia*) bark with 70% EtOH extraction (RCB) was performed on June 8th, 2016. Additionally, field tests of the other two residues resulting from extraction of ginger (*Zingiber officinale*) and magnolia bark (*Mongolia obovate* and *M. officinalis*) with 70% EtOH extraction (RG, RMB) was performed on May 8th, 2017. These field tests were carried out in Kyoto Institute of Technology, Kyoto, Japan. These residues were provided by Nippon Funmatsu Yakuhin, Co., Ltd. Mulch was prepared with thicknesses of 0, 1, 2, and 3 cm for RCB or 0 and 3 cm for RG and RMB on the soil surface. Weed species and their masses were measured at 3 months after the treatment.

The MeOH extracts were prepared from these residues by MeOH extraction, respectively. Phytotoxic compounds were isolated using silica gel flash column chromatography and identified by spectroscopic data. Phytotoxicity tests were performed using lettuce (*Lactuca sativa* cv great lakes) and Italian ryegrass (*Lolium multiflorum* cv wasefudo) seedlings.

The RCB mulch completely controlled various annual weeds, while did not affect the growth of some perennial weeds. Only *t*-cinnamic aldehyde was isolated and identified as a phytotoxic substance in the RCB MeOH extract. The RG mulch was muddy after a rain, as the results it was unsuitable for mulch. The RMB mulch also completely controlled various annual weeds. And then magnolol was isolated and identified as a phytotoxic substance from the RMB MeOH extract.

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### Evaluation of putative allelochemicals, cinnamic acid and an anthocyanin, in *Spiraea thunbergii* and *S. cantoniensis* using in vitro bioassay method of allelopathy, the protoplast co-culture with digital image analysis

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Strong inhibitory allelopathic activities of Spiraea thunbergii and its putative allelochemical, cis-cinnamic acid, and moderate activity of S. cantoniensis were reported by conventional bioassay methods. To know the cellular mechanism (s) of allelopathy, we introduced another bioassay method of allelopathy, protoplast co-culture method with digital image analysis (DIA-PP method), which quantitate the effects of protoplasts of test plants on the growth of recipient lettuce protoplasts, co-cultured in a 50 µL medium in a 96-well culture plate. Green leaves of aseptically grown seedlings of both Spiraea species, and purple leaves of S. cantoniensis, which anthycyanin-synthesizing gene was introduced, were investigated. Enzymatic conditions for protoplast isolation were optimized for each material. Effects of cis-, trans-cinnamic acid, and cyanidin glucoside were also quantitated. Numbers of non-spherically enlarged (cell wall formation stage) or divided protoplasts (cell devision stage) were counted under an inverted microscope, and Image J analysis of scanned digital image of yellow color accumulation in a 96-well plate was performed. Growth of lettuce protoplasts was described as % of control. Green leaves of S. thunbergii and purple leaves of S. cantoniensis and cyanidin glucoside strongly inhibited lettuce growth at cell division stage. The results were compared with those investigated using the same DIA-PP method.

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#### Phytochemical screening and allelopathic activity of ethanolic extract and solvent fractions of *Vernonia Amygdalina* Del. leaf

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Vernonia amygdalina Del. is a native African plant of the Asteraceae family that has been commonly known as bitter leaf due to its bitter taste. The possible medicinal use of V. amygdalina by wild chimpanzees was first described since 1989 and subsequent researches has unveiled that V. amygdalina contains a wide variety of bioactive compounds. However, there has not been any detailed research on allelopathic activity of this species. In 2017, the allelopathic potentiality of this plant was primarily evaluated by sandwich and dish pack methods at Fujii laboratory (TUAT). V. amygdalina showed significant strong inhibitory effect on lettuce radicle and hypocotyl elongation, compared to other local botanical species in Mekong Delta Vietnam. Therefore, the aim of this study was to continue investigating the phytochemical component and inhibitive activity of the extracts of V. amygdalina leaf on lettuce seeds. Dried leaves were extracted with 95% ethanol and then seperated by column chromatography using various solvents: hexane, ethyl acetate, n-butanol and water. The phytochemical analysis revealed the presence of secondary metabolites in crude extract, e.g. flavonoids, saponis, tannins, phenolics, steroids, alkaloids, terpenoids and cardiac glycosides. The ethyl acetate fraction showed the highest total phenolic content (74.83 mg QEA/g extract) and the highest flavonoid (63.62±1.11 mg RU/g extract), while the highest total tannin content was achieved in water fraction (1.77±0.02 %). The strongest inhibitory activity on the growth of lettuce roots was found in n-butanol fraction by 58.41%, yet hypocotyl elongation was not statistically different among separated fractions. At the concentration of 0.5 mg/mL, lettuce radicle and hypocotyl elongation were lowest by 73.26 and 78.29%, respectively. These results suggest that V. amygdalina can be an allelopathic candidate for further studies.

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# Species-specific allelopathic potential of Vietnamese tea (*Camellia sinensis*)

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The bio-herbicides isolated from plants are applied for weed control by replacing synthetic herbicides or can be discovered as lead chemicals for synthesis of a new herbicide. It was reported that tea plant (*Camellia sinensis*) has a significant allelopathic effect from tea extract and residues. However, each kind of weeds or crops has different sensitivity to inhibitory compounds extracted from allelopathic plants. In this study, we evaluated allelopathic potentials of aqueous extracts of green and oolong tea from Vietnam on growth of twelve crop and weed species. For the evaluation of plant germination, 1 ml of each extract (50, 75 and 100 mg/ml) was added to a sheet of filter paper ( $\phi$ 42.5 mm) in a 50 mm Petri dish, and then ten seeds of each tested species were placed on the filter paper, and incubated in dark condition at 22°C for n-days. For the evaluation of plant growth: 700 µl of the crude extracts of tea leaves at concentrations of: 1, 2, 4, 6, 8, and 10 mg/ml were added to the filter paper. Then five lettuce seedlings were put on the filter paper, and incubated in dark at 22°C for 3 days. The length of radicle and hypocotyls/coleoptiles of lettuce seedlings were measured on the third day.

The resulst showed seed germination of all the tested species was inhibited by green and oolong tea extract at concentration 100 mg/ml, but inhibitory effect induced by extract was not observed on the germination at both rice cultivars. Based on EC50 value, green tea extract significantly inhibited the growth of the weeds such as: timothy, white clove and red clover by possible presence of high caffeine content. By contrast, extract had a slight effect on the growth of rice, carrot and hairy vetch at the tested concentrations (4, 6, 8, 10 mg/ml). The observed differences in the response (germination, radicle and hypocotyls/coleoptiles elongation) were possibly due to the difference in seed size and seed coat permeability which are responsible for caffeine uptake. Our findings revealed that extract from green tea significantly inhibited the weed species than oolong tea, suggesting a new weed control potential.

#### Evaluation on the allelopathic potentials of Malaysian plants, and identification of goniothalamin as a potent allelochemical from *Goniothalamus andersonii* J. Sinclair

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Evaluation on the allelopathic potentials of 145 plants was conducted by using the sandwich method. The bark of Goniothalamus andersonii exhibited the greatest inhibition rate of 81% on the radicle growth of lettuce seedlings at 10 mg dry weight. Goniothalamin was identified as a potent allelochemical from the bark by bioassay-guided purification. This compound showed the  $EC_{50}$  values 50 and 125 µmol L<sup>-1</sup> for both radicle and hypocotyl growth of lettuce seedlings, respectively. Soil incorporation with the bark powder of G. andersonii was conducted to assess the plant growth inhibitory activity of this plant against lettuce (Lactuca sativa L.), cucumber (Cucumis sativus L.), white clover (Trifolium repens L.) and perennial ryegrass (Lolium perenne L.). Various concentrations of the bark powder incorporated into soil have resulted in the reduction on the growth rate, fresh and dry weight of those tested plants parallel with the increasing concentration of bark powder treated. At the highest concentration of 2%, the growth of perennial ryegrass was strongly inhibited after 14 days of sowing (95%). The strong detrimental effect on this invasive perennial ryegrass in particular indicates that the bark of G. andersonii could be very beneficial as a bio-herbicide for practical weed management.

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#### Identification of allelopathic substances of desert plant Sea-buckthorn

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Leaves, fruits and other parts of 22 plants from desert plants of Inner Mongolian Autonomous Region were examined by the "Sandwich Method" which is widely used to assay allelopathy of plant parts. The results of the test are as follows: Hippophae rhamnoides (Sea Buckthorn) 96%, Artemisia sieversiana 94%, Salsola collina 92%, Artemisia annua 87%, Platycodon grandiflorus 85%, Salix cheilophila 83%, Agriophyllum squarrosum 83% (in order from strong to weak). Strong activity was observed in most of the species. As a result of the bioassay by the dishpack method, which tests the allelopathy of plant volatile substances, strong activity was observed in the order of Artemisia annua (Sweet annie) 85%, Sabina vulgaris 55%, Artemisia ordosia (strong to weak) 26%.

The strongest Sea Buckthorn fruit inhibited the growth of the recipient plant lettuce by 96% at 10 mg-dry weight/10 mL agar. Sarcosine and asparagine were detected as candidates for potential allelochemical as a result of GC-MS analysis of water extract of Sea Buckthorn fruit.

In the test of volatile substances of Sweet annie, the growth of the recipient plant lettuce was inhibited and 1, 8-cineole was detected as the major compound by GC-MS analysis.

# The possible role of allelopathy in invasive succession of *Papaver dubium* L.

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*Papaver dubium* L. is an annual alien species in Japan that was first time found in Setagaya, Tokyo in 1961. Since then, it has been widely spread across Japan, especially in central and southern areas. Allelopathy has high contribution to invasive properties. Therefore, it is essential to know their ecological characteristics, especially allelopathy.

In this experiment, in order to determine the possible role of allelopathy in the spread of *P. dubium* in Japanese agroecosystem, Sandwich method (SW), Plant Box method (PB) and soil incorporation test were adapted and its inhibitory activity on elongation and dry weight of lettuce seedlings were measured. Moreover, fresh leaf and root were extracted with aqueous methanol to determine the  $EC_{50}$  on lettuce radicle growth. HPLC was used to analyse the chemical composition in the aqueous methanol extract of the *P. dubium*.

*P. dubium* showed high inhibitory activity by SW (Leaf 50mg: 82%, Root 50mg: 56%), PB (63%) and the soil incorporation test (EC<sub>50</sub>: 1.7% (w/w)). The extracts of fresh leaf showed inhibitory activity by increasing concentrations (EC<sub>50</sub>: 30mg/mL). The HPLC analysis revealed that the fresh leaf and the root of *P. dubium* contain high concentration of berberine (Leaf: 13.2µg/mg, Root: 13.3µg/mg). Pure compound of berberine showed inhibitory activity by increasing concentrations (EC<sub>50</sub>:  $25\mu$ g/mL). The total activity of the berberine was estimated 0.53. Based on the present results, It is possible that berberine play some roles in invasive succession of *Papaver dubium* L.

#### Allelopathic and antimicrobial study of Acacia modesta and Buxux papillosa available in District Hangu, KPK, Pakistan

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The current study was to asses' allelopathic activities and antimicrobial potential of Acacia modesta Wall and Buxus papillosa C. K. Schneider. A laboratory based studied was made to investigated the allelopathic potential of A. modesta and B. papillosa leaves and roots on Lettuce seed (Lactuca sativa L. v. Great lakes 366). Results were significant which showed that 100 g/L water extraction of leaves of both the plants having high allelopathic potential to inhibit the seedling growth of lettuce seeds. Roots water extractions of both the plants induce seedling growth of tested plant. The in vitro antimicrobial investigations of crude methanolic extracts of A. modesta (bark and root) and B. papillosa (leaves, stem and root) plants were analyzed against five pathogenic bacteria including one Gram positive Bacillus subtilis, four Gram negative i.e. Escherichia coli, Klebsiella pneumonia, Xanthomonas campestris and Agrobacterium tumefaciens using disc diffusion method. We obtained significant results from this experiment. It was observed that K. pneumonia was the most resistant bacterium to all extracts. Present study concluded that these plants are good sources of antibacterial compounds for the curing of many pathogenic diseases. These results confirm strong allelopathic and antimicrobial potential of these both of these candidate species.

#### How to promote the growth of young Japanese Pear trees with the cyanamide and establish the system to determine when we should use the cyanamide

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Cultivation of Japanese pear, which is a major fruit in Japan, has been expanding in East Asia. Japanese pear is necessary to replant in every 30 years, but the growth of replanted trees were usually suffered from soil sickness syndrome. Cyanamide, a putative allelochemical from hairy vetch, has anti-microbial, insecticidal and herbicidal activity, and also used practically to promote the flowering of grape.

In order to establish the method for culturing trees of Japanese Pear 'Kosui' growing up in a short period by applying the cyanamide, which is the chemical used for stabilizing the flowering, we carried out the following verification test and system development.

• In case the cyanamide was sprayed on the nursey trees in orchard field, the sprouting rates of leaf bud at a year and two were 94.0% and 96.5% respectively, both of which were significantly greater than no-treatment trees (controls). The number of shoots and total elongation of shoots were greater than the controls at three years.

• In case the cyanamide was sprayed on an aging nursery in the replanted orchard field, both sprouting rates of leaf bud and the number of shoots were significantly greater than controls, as an aforementioned example. Therefore, we consider that cyanamide could be used not only for stabilizing the flowering, but also for facilitating the early bearing of replanted orchard field.

• We developed a system which calculates the appropriate timing for spraying the cyanamide by downloading the temperature data by AMeDAS(Japan Meteorological Agency official website) into a personal computer automatically, and then calculates the DVI (developmental indexes) according to the growth forecast model.

#### Identification of allelopathic compound of fenugreek and its allelopathic potential against some weeds under laboratory and greenhouse conditions.

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Fenugreek is one of the promising medicinal herbs. It has potent allelopathic activity, and the activity is confirmed through laboratory and greenhouse bioassay in this study. laboratory bioassay tests have been carried out by sandwich method and plant box method with lettuce seeds. As for sandwich method the inhibition percentage of lettuce radicle by fenugreek leaves (91%) was greater than its seeds (60%). Regarding the result of plant box method showed that the fenugreek root exudates have allelopathic effect which able to suppress lettuce seedling growth by 86.1%. To identify fenugreek allelochemicals, dried leaves were extracted by distilled water then partitioned with different solvents. Chloroform layer showed the highest biological activity. Isolation step has been performed using C18 and HLB columns, inhibitory activity was found in 80% methanol fraction. Further cleanup has been done using Preparative- HPLC. The EC<sub>50</sub> values of this fraction have been evaluated against some plant species namely: lettuce (4.8 ppm), Alfalfa (19.4 ppm), white clover (28.8 ppm), Verdolaga (31.7 ppm), Centipede grass (37.75 ppm), Timothy (3.5 ppm). This fraction has been identified by GC-MS instrument as dihydroactinidiloide compound. Regarding greenhouse experiment, three levels of fenugreek leave powder (0.5, 1 and 1.5 %) incorporated with soil. Two obnoxious weeds, wild oat and dandelion have been used in this test. The results showed that both of 1 and 1.5% of fenugreek leaves powder caused totally inhibition on germination process of dandelion. While wild oat, fenugreek leaves caused significantly decrease in root and shoot length. Knowledge of fenugreek allelopathy will offer possibilities for ecological management of weeds under field conditions.

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#### **Evaluation of the allelopathic activity of Sudanese plants for weed control in** *Sorghum bicolor*

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Sudan is a major Sorghum producing country; however, its production has been hindered by the presence of serious invasive weeds (Azhari, 2000; Badi et al., 1990). Intensive use of chemicals to control weed species has led to increase weed resistance to pesticides (Michael et al, 2005). One of the effective alternative ways in weed control is allelopathy, i.e. using of natural chemicals from plants to minimize the dependency on synthetic herbicides. Therefore, our research aims to assess the allelopathic potential of fifty-nine plants originated from Sudan and to identify allelochemicals and their specific activity. Allelopathic potential of soluble and volatile compounds was evaluated using Lactuca sativa as receptor plant according to (Fujii et al., 1994; Fujii et al., 2000). Identification of potential allelochemicals was done using GC-MS and HPLC; the contribution of an individual compounds into the total inhibitory activity was determined by (Golisz et al., 2007) and (Mishyna et al., 2015). The top list of Sudanese plants with the strongest allelopathic activity through exudates includes Lawsonia inermis (leaves, powder), Hibiscus sabdariffa (fruit), Adansonia digitata (fruit), and Balanites *aegyptiaca* (leaves). The highest suppression of the hypocotyl growth of lettuce seedlings by volatiles compounds was observed for Terminalia brownie (wood), Euphorbia hirta (leaves), Diospyros mespiliformis (leaves), Adansonia digitata (leaves), Corchorus olitorius (leaves), and Hibiscus sabdariffa (fruits). Moreover, 1-decyne was identified as the main volatile compound naturally released from dried A. digitata leaves and responsible for the allelopathic activity expressed by volatiles from baobab leaves against lettuce growth. The allelochemical lawsone has been identified in Lawsonia inermis leaves by HPLC and mainly contributed to the total allelopathic activity of soluble exudates from *Lawsonia inermis* leaves. Therefore, plant species from Sudan showed a high allelopathic potential and can be used for future organic herbicide development.

# L-DOPA in *Vicia faba* sprouts and gene that produce L-DOPA

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L-DOPA plays two roles of treatment of Parkinson's disease and allelopathy. Parkinson's disease is commonly treated by ingestion of L-DOPA. In plants, L-DOPA has allelopathic activity to inhibit radicle growth, and the use of weed control has attracted attention. L-DOPA is made from tyrosine by tyrosine hydroxylase and tyrosine hydroxylase is classified in 14-3-3 protein family in eukaryotes. However, genes that produce L-DOPA in plants have not been elucidated. If it is clarified, it is expected to contribute to the development of medical and agricultural aspects. In this study, the correlation between the content of L-DOPA and the expression level of L-DOPA genes in sprout, the purpose of cloning 14-3-3d sequence of Vicia faba produced as L-DOPA genes The experiment was carried out.Previous studies have obtained data suggesting a change in L-DOPA content in Vicia *faba* sprouts and have experimented with the hypothesis that the expression of L-DOPA genes also fluctuates. In this study, I measured the amount of L-DOPA contained in the Vicia faba sprout HPLC. As a result, it was found that the content of L-DOPA increased about 2-fold from 7th day to 14th day after seeding. Next, BLAST search was performed based on the sequence of broad bean 14-3-3 protein to obtain the 14-3-3 amino acid sequence and cDNA sequence of *Vicia faba*, then I called it *vf14-3-3d* sequence. Primers for cloning and expression were designed based on the information of this base sequence. We succeeded in amplifying the DNA fragment by PCR with the ORF primer using hydroxylase cDNA and genomic DNA for vf14-3-3d.

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#### **Evaluation of 91 Chinese medicinal plant species for allelopathic potential**

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In plant kingdom allelopathy is an essential ecological factor as well as phytotoxic in medicinal plants (Samanta et al., 2011). Medicinal plants contain a relatively large number of secondary metabolites and allelopathic activities have been frequently found in those plants (Fujji et al., 1990). This study focused on Chinese medicinal plants, which are mainly distributed in northeastern and southeastern of China. The objectives of this study were: (1) Screening of allelopathic activity of medicinal plants in China. (2) Evaluation of allelopathic activity of plants candidates for weed control. (3) Identification of allelochemical(s) from potential allelopathic plant species.

For the materials, 91 species medicinal plants were collected from Xinjiang Institute of Ecology and Geography and Beijing Hospital of Traditional Chinese Medicine. Plant materials were screened using the Sandwich method (Fujii et al., 1994) and Dish Pack method (Fujii et al., 2000) to evaluate their allelopathic effect on the growth of lettuce (*Lactuca sativa*). Phytotoxic activity experiment was used to determine allelopathic activity on crops and weed species. Cotton swab method was used to determine allelopathic activity of volatile allelochemical (Mishyna et al., 2015). Identification and quantification of potential volatile allelochemical(s) were done by GC-MS.

As the results, fruits of *Illicium verum* showed significant inhibitory acitivity in both Sandwich and Dish-pack method. The fruits of the Chinese star anise (*Illicium verum*) contain SA to the extent of 17.14% on dry wt. basis and this is now the main source for commercial production of SA (Jagat Chandra Borah, 2015). According to the EC<sub>50</sub> result, it was concluded that radicle growth inhibition of lettuce caused by crude extract of star anise could be explained by contribution of biological activity of shikimic acid. GC-MS analysis of *Illicium verum* (0.5g) showed anethole could be the major volatile compound in this plant by comparing with database. The result of cotton swab method showed the strong growth inhibition of authentic anethole and star anise powder on lettuce respectively. The result indicated that anethole has a strong inhibitory activity and has a big contribution in allelopathic activity of star anise. It also indicated that EC50 of anethole in head space of star anise powder is almost similar to that of authentic anethole, so anethole could explain as principle inhibitory volatile in head space of star anise.

In conclusion, fruits of *Illicium verum* showed significant inhibitory activity in both sandwich and dish-pack method. Two major compounds, shikimic acid and anethole in star anise have showed strong inhibitory activities on crops and weed species. They could be the putative allelochemicals in *Illicium verum* and could be utilized in weed management and sustainable agriculture.

#### **CREST project and future dream for research** -Elucidation of rhizosphere chemical world for the regulation of crop robustness-

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Crops such as soybean and tomato can only be grown to about half of their potentially achievable yield due to various problems related to soils. In this research, we develop a multimodal sensor device to diagnose minerals and metabolites in the rhizosphere, which has strong influence on the crop growth. We identify biomarkers for robustness by integrating data from sensors and omics and analyzing in multimodal deep learning. We verify biomarkers under fluctuating environment to achieve crop robustness for the increase of production.

For allelopathic research, we are starting to use hairy vetch as green manure plant for soybean growth. Yield of soybean after hairy vetch usually 10 to 20 % higher than control and quality of bean also increase. Metabolomic analysis in combination with other factors will explain the important factor in the soil.



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