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Allelochemical effects of aromatic species intercropped with coffee (*Coffea arabica* L.) in Puebla, Mexico

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Allelochemical effects of aromatic species intercropped with coffee (*Coffea arabica* L.) in Puebla, Mexico

Over 30 million coffee growers all over the world face starvation due to low international coffee prices. The impact is very acute in Latin America, which accounts nearly 65% of world coffee production. The current crisis seems to be shaped by changes in consumer preferences as well as low production phenomenon due to caffeine accumulation in coffee plantation soils as a result of decades of monoculture. To turn the present crisis around, a successful strategy for agricultural diversification is required. The production of organic coffee or the improvement of its quality, as well as the diversification of production through the introduction of tropical crops, vegetables, fruits or aromatic plants can increase producer's survival chances in a self-sustainable way. In response to the crisis, some coffee growers have begun to diversify their coffee plantations with intercalated cultivations of aromatic plants. Herbs are profitable crops and adaptable to the environmental conditions of coffee regions. Due to the complexity of the natural interaction under intercropped systems and the potential allelopathic effects between coffee and aromatic herbs, some questions need to be clarified before this alternative production system can be recommended to growers. Accumulation of caffeine in a soluble form in the soil is regarded as one reason for "low production" and degeneration by auto toxicity of coffee plantations. The use of aromatic species with the ability to take-up and accumulate caffeine may be a way to diminish the toxic levels of this alkaloid and increase coffee production. In this present study, the potential uptake of caffeine by spearmint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), sage (*Salvia officinalis* L.), and oregano (*Origanum vulgare* L.) and the allelopathic effects of these herbs on physiological parameters in coffee (*Coffea arabica* L.) were investigated. Two ecological conditions in rural area of Puebla State, Mexico (2004-2005), as well as laboratory bioassays in Bonn, Germany (2006) were evaluated, to validate the hypothesis that intercropping herbs in coffee production systems is a possibility to attenuate the coffee crisis while positively stimulating coffee plant growth and cup quality, diminishing caffeine content in the soil. To summarize the results: 1. Intercropping sage, spearmint, basil and oregano stimulate the plagiotropic growth of *Coffea arabica* plants most effectively in young production systems by still unknown mechanisms. 2. Volatiles from essential oils induce stomata opening in coffee leaves, which may have a positive influence on the CO_2 fixation and increase of photosynthetic activity when no limiting factors are present. 3. Aromatic species, principally sage and oregano absorb caffeine and can contribute to a diminishing of the caffeine contamination of the soil. 4. Cup quality is improved with spearmint, basil and sage as intercrops, but mechanisms of action are unknown and further research remains to be done. 5. Finally coffee growers can stabilize their income situation and their social condition by offering aromatic plants to the local markets produced during the no-harvest period of coffee (April –November) in between coffee rows. An alternate and appropriate packing system could be under modified atmospheres, increasing shelf life of the herbs up to 100% more than traditional management.

Allelochemische Einflüsse von Gewürzpflanzen im Zwischenanbau mit Kaffee (*Coffea arabica* L.) in Puebla, Mexiko

Heute leiden mehr als 30 Millionen Kaffeeanbauer unter dem niedrigen Weltmarktpreis. Besonders hart trifft es die Kaffeeanbauer in Lateinamerika, die etwa 65 Prozent des Weltaufkommens erzeugen. Diese Krise basiert besonders auf veränderten Konsumgewohnheiten und wird verschärft durch geringe Erträge, die auch durch die jahrzehntelange Monokultur von Kaffee und damit verbundene Koffeinanreicherungen im Boden verbunden ist. Eine Lösung der Krise wird besonders unter Einbeziehung neuer Strategien zur nachhaltigen Diversifizierung traditioneller Kaffeeanbauregionen mit standortspezifischen neuen Kulturen angestrebt. Dazu gehören sowohl der organische Anbau von Kaffee als auch tropische Obstkulturen, der Gemüseanbau und der Zwischenanbau von Gewürzen. Der Zwischenanbau von Gewürzen in bestehenden Kaffeepflanzungen wurde empirisch von einigen Kaffeeproduzenten in verschiedenen Ländern begonnen. Dazu wurden standortsbezogen geeignete, hochprofitable Gewürzpflanzenarten ausgewählt. Die Komplexität natürlicher Wechselwirkungen von Gewürzpflanzen im Zwischenanbau mit Kaffee und das Potenzial allelopathischer Effekte zwischen Kaffee und Gewürzpflanzen sind bisher nicht umfassend geklärt und müssen vor der weiteren Anbauausdehnung wissenschaftlich bearbeitet und geklärt werden. Allgemein bekannt ist die Anreicherung löslicher Formen von Koffein im Boden und damit ausgelöste Ertragsminderungen durch die Degeneration der Kaffeepflanzen basierend auf autotoxischen Einflüssen. Die bewusste Nutzung von Gewürzpflanzenarten zur Minderung von Koffeingehalten im Boden durch die Aufnahme dieses Alkaloids könnte eine interessante Lösungsvariante darstellen. Die vorliegende Dissertation beschäftigt sich mit dem spezifischen Aufnahmevermögen von Koffein durch Pfefferminze (*Mentha piperita* L.), Basilikum (*Ocimum basilicum* L.), Salbei (*Salvia officinalis* L.), und Oreganum (*Origanum vulgare* L.) und den allelopathischen Effekten dieser Pflanzenarten auf physiologische Parameter von *Coffea arabica*. Die Versuche wurden auf zwei Kaffeestandorten in Puebla, Mexiko (2004-2005), und unter Laborbedingungen an der Universität Bonn (2006) durchgeführt, um die Hypothese zu prüfen, dass Gewürzpflanzenarten im Zwischenanbau mit Kaffee in der Lage sind, diese aufgrund der Verminderung des Koffeingehaltes im Boden in ihren wachstums- und ertragsbildenden Parametern zu stimulieren und die Kaffeequalität zu verbessern. Zusammenfassend kann festgestellt werden: 1. Zwischenkulturen mit Salbei, Pfefferminze, Basilikum und Origanum stimulieren das Wachstum plagiotroper Zweige von *C. arabica* besonders in Jungpflanzungen, ohne bisher diese Mechanismen bestimmen zu können; 2. Die Applikation von essentiellen Ölen dieser Pflanzenarten bewirken die Öffnung von Stomatazellen der Kaffeeblätter und haben positiven Einfluss auf die CO_2-Bindung und photosynthetische Aktivitäten, wenn keine limitierenden Faktoren existieren; 3. Gewürzpflanzen, insbesondere Salbei und Origanum absorbieren Koffein und können zu deren Abbau im Boden beitragen; 4. Die Tassenqualität von Kaffee wird verbessert durch den Zwischenanbau von Pfefferminze, Basilikum und Salbei, ohne dass die speziellen Mechanismen dafür geklärt werden konnten; 5. Das wirtschaftliche Einkommen von Kaffeeproduzenten lässt sich durch Zwischenkultur von Gewürzpflanzen in der ertragsfreien Kaffeeperiode (April bis November) verbessern und sollte durch geeignetes Nacherntemanagement und Verpackungspraktiken mit modifizierter Atmosphäre, die die Haltbarkeit der Frischprodukte um 100 % verlängern, ergänzt werden.

Efectos aleloquímicos de plantas aromáticas intercaladas con café (*Coffea arabica* L.) en Puebla, México

En el momento, cerca de 30 millones de cafetaleros en todo el mundo enfrentan una situación insostenible debido a los bajos precios internacionales del café. El impacto es muy intenso en Latinoamérica, en donde se encuentra alrededor del 65 % de la producción mundial de café. La presente crisis parece empeorar por los cambios en las preferencias de los consumidores así como también el fenómeno de baja producción debido a la acumulación de cafeína en terrenos cafetaleros como resultado de décadas de monocultivo. Para revolver la presente crisis, se requiere una estrategia exitosa de diversificación agrícola. La producción de café orgánico o la mejora de su calidad, así como también la diversificación de producción a través de la introducción de cultivos tropicales, verduras, frutas o plantas aromáticas, pueden aumentar las oportunidades de supervivencia del productor en una forma auto-sostenible. En respuesta a la crisis algunos cafetaleros han comenzado a diversificar sus cafetales con cultivos intercalados de plantas aromáticas. Las hierbas aromáticas son cultivos rentables y adaptables a las condiciones ambientales de regiones cafetaleras. Debido a la complejidad de la interacción natural bajo los sistemas intercalados y los efectos alelopáticos potenciales entre café y plantas aromáticas, algunas preguntas deben ser aclaradas antes de que este sistema alternativo de producción pueda ser recomendado a los cultivadores. La acumulación de cafeína en una forma soluble en el suelo es considerada como una razón para la degeneración y "baja producción" por auto-toxicidad del café. El uso de especies aromáticas con la habilidad para absorber y acumular cafeína puede ser una forma para disminuir los niveles tóxicos de este alcaloide y aumentar la producción de café. En este estudio el potencial de absorción de cafeína por menta (*Mentha piperita* L.), albahaca (*Ocimum basilicum* L.), salvia (*Salvia officinalis* L.) y orégano (*Origanum vulgare* L.), y los efectos alelopáticos de estas plantas sobre los parámetros fisiológicos en café (*Coffea arabica* L.) fueron investigados. Dos condiciones ecológicas en el área rural del Estado de Puebla, México (2004-2005), así como también ensayos biológicos de laboratorio en Bonn, Alemania (2006) fueron evaluados para validar la hipótesis de que las hierbas intercaladas en sistemas de producción cafetaleros son una posibilidad para atenuar la crisis y al mismo tiempo estimular positivamente el crecimiento de las plantas de café y la calidad de la taza, disminuyendo el contenido de cafeína en los suelos. Para resumir los resultados: 1. Al intercalar salvia, menta, albahaca y orégano se estimula el crecimiento plagiotrópico de *Coffea arabica* con mejores resultados en nuevos sistemas de producción involucrando mecanismos aún desconocidos. 2. Volátiles de aceites esenciales inducen a la apertura estomatal en hojas de café, lo cual puede tener un efecto positivo en la fijación del CO_2 y el incremento de actividad fotosintética cuando no existan factores limitantes. 3. Las especies aromáticas, principalmente salvia y orégano absorben cafeína y pueden contribuir a una disminución de la contaminación de cafeína del suelo. 4. La calidad de la taza es mejorada al intercalar salvia, menta y albahaca, pero los mecanismos de acción son desconocidos y se requiere más investigación sobre el tema. 5. Finalmente los cafetaleros pueden estabilizar su situación de ingresos y su condición social ofreciendo plantas aromáticas sembradas en medio de las filas del café durante períodos de no cosecha de café (abril – noviembre) y comercializarlas en los mercados locales.

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1 INTRODUCTION

1.1 Present day coffee constrains

Coffee has long been one of Latin America's most lucrative and extensively cultivated cash crops. Its production and processing is a significant source of revenue and employment in rural communities for many countries. Presently, coffee growers all over the world face starvation due to the international coffee crisis. Nowhere is the impact more acute than in Latin America, which accounts for nearly 65 percent of world coffee production (USDA, Foreign Agricultural Service; Figure 1).

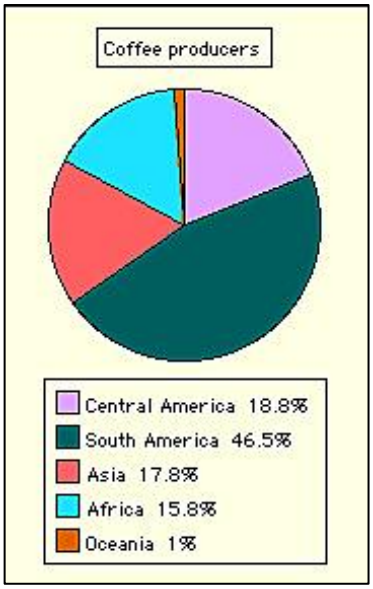

Figure 1: Contribution by region to coffee world market

Many factors, including overproduction have caused the present situation. Brazil has increased its production by 20 % over the last 10 years and Vietnam has joined the market as a major new producer (Wilson, 2001).

The International Agreement on coffee was cancelled in 1989, thus terminating a pact of quotas that provided the instruments for regulating the international market. Prices on the world market, which averaged around 120 US cents/lb in the 1980s, are now around 50 cents, the lowest in real terms for 100 years (International Coffee Organization; Figure 2).

These low prices coincide with a change in consumer preferences which tend towards increased demand for organic products and new specialty coffees. The large number of hands coffee passes through and the oversupply of coffee on the world market (partially due to relatively new exporting countries, such as Vietnam) have contributed to diminish

farm income. While coffee prices have reached an all time low, large profits are still made by coffee traders, though farmers usually see but a small percent of these profits[1].

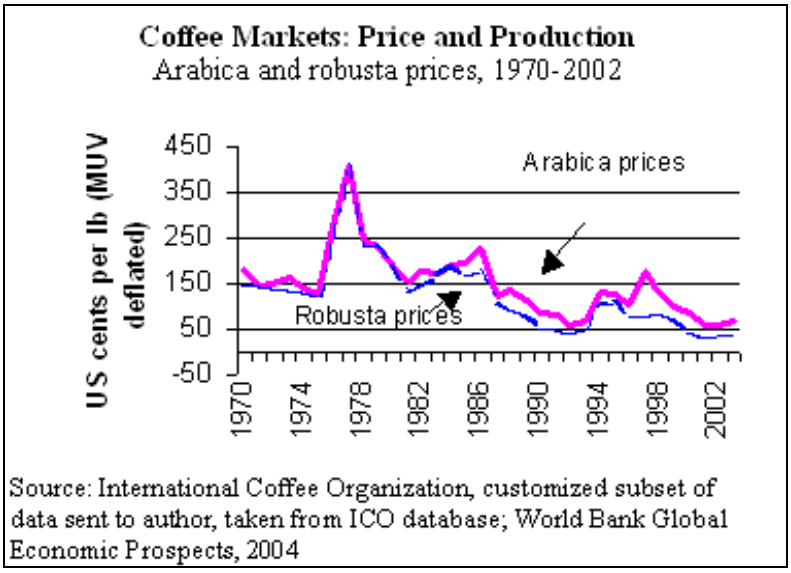

Figure 2: Dynamic of coffee prices in the past three decades

1.2 Environmental implications

Environmental issues are the last priority for many farmers struggling to cope with the coffee price crisis. Meanwhile, some new environmental damage has increased, such as the destruction of shade-tree systems, followed by decreasing biodiversity and the destruction of ecosystems and natural habitats. Some of the key environmental problems arising from the coffee crisis are as follows:

[1] Additional information on the coffee crisis in Latin and Central American countries can be obtained from a variety of sources including official information from ICO (International Coffee Organization) on the following web-sites:

www.ico.org/crisis

www.eldis.org/csr/coffee.htm

www.coffeehabitat.com/2006/02/the_coffee_cris.html

www.oxfamamerica.org/resources/files/crisis_continues_summary

Caffeine accumulation in coffee systems

The purine alkaloid caffeine is a biologically active compound found in members of the *Rubiaceae* family that contribute to allelopathic and auto-toxic effects appearing in old coffee plantations. Caffeine released from the tree's own litter over the years and accumulated in the vicinity of roots is leached out into aqueous media, suggesting storage in a soluble form in the soil. Since most coffee roots develop in the upper soil layer, immediately under the tree's own litter, auto-toxicity can be manifested in soils of long establishment of monoculture regarding one reason for "low production phenomena".

Introduction of new crops and abandonment of farms

Introducing new crops that are not adapted to the slopes present in the coffee growing regions of the world causes serious erosion problems. Furthermore, abandoning a coffee plantation and leaving coffee cherries un-harvested attracts pests and can cause serious plagues and infestations of pests in subsequent years, worsening the situation of neighbour plantations.

Destruction of the shade-tree system

The coffee crisis drives traditional coffee producers to cut down and sell their shade trees as timber or firewood. This forest cover provides habitat for migratory birds and functions to protect the watershed from soil erosion. Shade coffee farms throughout Latin America have been cleared and often replaced with subsistence agriculture (peanut, corn, beans), pasture, or where some farmers can no longer afford to grow quality coffee new houses, resorts and hotels are constructed.

Increase of illegal crop areas

Falling coffee prices push farmers to plant illegal crops. Coffee growers in some conflict regions have scrapped their coffee plants and shade trees in favour of coca and poppy, increasing the area under illegal crops. Government eradication programs are based on spraying the planted areas with herbicides (glyphosate), but the ecological damage is widespread because of inefficient and uncontrolled aerial application.

Increase of high input technologies

The implementation of the new technologies has altered the natural ecosystem, forcing coffee producers to continually increase the amount of agrochemicals they use. These practices not only damage the environment, but have also undermined the cost-competitiveness of the coffee enterprises themselves.

1.3 How to confront the crisis

The current coffee crisis in Latin America is primarily an issue of improving the competitiveness of smallholder and medium sized agricultural producers within the global economy with new market structures and new challenges. Consumption patterns and trends are at the root of the decisions of whether to plant, what to plant, how to mill and when to bring coffee to the market. While the coffee grower has to focus on the market, the market is focusing on the grower. Issues like traceability and certification are increasingly capturing consumer's attention and directing their eyes to what happens on farms. Is coffee produced in a socially and environmentally responsible way?

Some components to confront the coffee crisis include investment in social services (health, education, transportation) complementing with light industry and other profitable activities that can be introduced in a sustainable and environmental friendly way.

The above mentioned factors call for new strategies, the centrepiece of which must be sustainable development of the rural economy. To manage the competitive transition of the coffee sector in Latin America, the following strategies have been adopted:

- ✓ Sustainable enhance coffee grower's efficiency and income in regions with comparative advantages for quality coffee, such as adequate altitude.
- ✓ Develop value added products (organic and speciality coffees) for suitable fare trade markets.
- ✓ Diversify production with high value crops as alternate income sources during the no harvest period (April - November).
- ✓ Convert coffee production regions where high quality coffee cannot be produced into production areas for other crops or into non-agricultural areas.

Under these circumstances, coffee producers have two options: to stay in the coffee business and change their attitude towards production and the environment or to exit the business. For those who stay, two strategies can be followed: to prune the trees and wait two to three years to see if the market recovers, and/or to increase the quality of their coffees and diversify their agricultural production.

The primary goal of this research is to provide alternatives that will allow coffee growers to keep the farm as an agricultural enterprise. As a secondary goal, the alternatives should help the growers to be self-sufficient and less depends on coffee prices. Alternatives should also aim to employ displaced coffee labourers and should favour the use of idle land; both practices are profitable and environmentally sustainable. These new alternatives need to be developed principally during the no harvest period (April - November) and should be short-term alternatives that complement the coffee plantation activity (Figure 3).

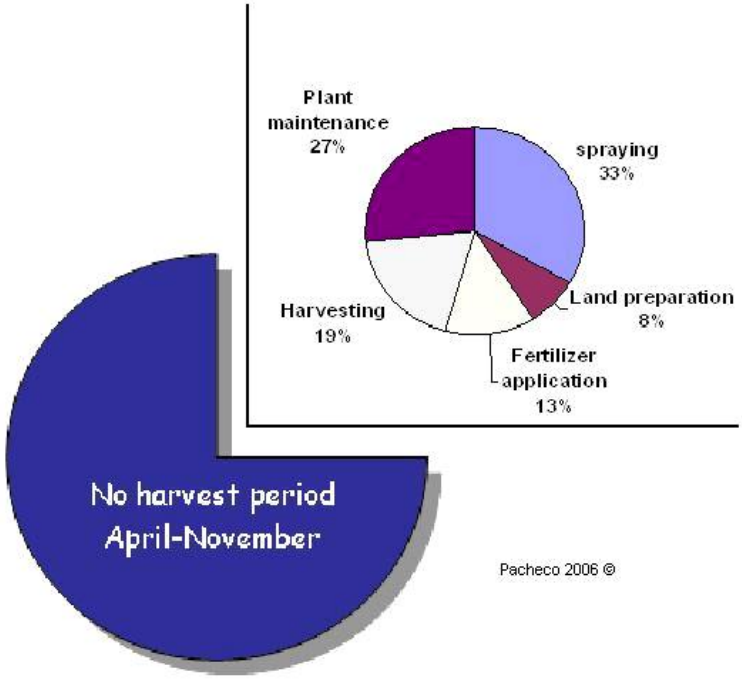

Figure 3: Distribution of labour during the year in coffee production systems

The situation is especially critical, between April and November (no harvest period) because most coffee producers are smallholders living in remote rural areas. They depend on the cash income from their own harvest and temporary picking work for survival. A crisis in the sector creates social imbalances, instability and accelerated migration to urban areas.

The coffee growers that confront the crisis have begun to diversify their coffee plantations with intercalated crops, such as timber, ginger (*Zingiber officinale* L.), vanilla (*Vanilla planifolia* L.), pepper (*Piper nigrum* L.), exotic flowers, etc. An interest in aromatic plants has been generated in the last years by the planter's community and the actual demand of these profitable products in the international market.

Aromatic plants have been used as raw materials for extraction of essential oils, for the flavour and fragrance industries as well as spices, herbs and traditional medicines, pharmaceuticals, cosmetics, botanical pesticides, insect repellents and herbal tea/drinks. Intercropping aromatic plants provides some advantages such as weed control, nutrient recycling, low-external input farming and extra income (Chou, 1986; Fisher, 1986; Rizvi and Rizvi, 1987; Pohlan *et al.*, 2003). The technical knowledge needed to produce good quality herbs is basic and the possibility of introducing them into the environmental conditions under coffee systems is high.

1.4 Aims of the research

1.4.1 General Hypothesis

1. Intercropping spearmint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), sage (*Salvia officinalis* L.), and oregano (*Origanum vulgare* L.) in coffee (*Coffea arabica* L.) production systems is a suitable environmentally friendly alternative which provides a potential detoxification of caffeine through uptake by aromatic plants. It may diminish toxic accumulation of the alkaloid in old coffee plantations, increasing production and generating extra incomes to coffee growers in crisis

2. Volatile essential oils from herbs (spearmint, sage, oregano and basil) may have a positive influence on vegetative growth and quality of coffee, as intercropped herbs are known to affect the leaf surface of surrounding plants, by potential allelopathic interactions.

1.4.2 General objectives

1. Quantify caffeine uptake and release capacity of herbs in coffee production systems. Test which factors affect caffeine dynamics, define time and quantity and physiological response to the alkaloid for each aromatic species.

2. Evaluate the participation of volatile essential oils in plant-plant relationships and possible targets in plant physiology, such as growth stimulation and quality improvement of coffee.

3. Define suitable aromatic species for intercropping in commercials coffee production systems under different ecological conditions; determine yield, post-harvest management and if modified atmosphere is an adequate packaging system for herbs.

4. Determine if caffeine and other compounds released by coffee may have a negative effect on yield of aromatic plants over the time.

5. Identify the potential effect of essential oils on growth and yield of vegetables like zucchini and beans.

2 CONCEPTUAL FRAMEWORK

2.1 Establishment of herbs in coffee systems

Aromatic plants grow throughout the Subtropical and Mediterranean areas and its cultivation is extended to the tropics countries lying between 45 degrees N and 35 degrees S and up to an altitude of 2500 meters above sea level (m. a. s. l.) These crops can be grown successfully in places receiving a minimum rainfall of 1.250 mm/year. The rainfall should be well distributed well during the flowering and pegging of the crops. The total amount required for preparatory cultivation is 100 mm; for sowing, 150 mm and for flowering and pod development an evenly distributed rainfall of 400-500 mm are required (Bareño, 2006).

The use of idle space among rows of coffee gives a suitable, safe and sustainable space to grow fresh herbs. The rows to be sown need to be at least 2 m wide and oriented East-West with a maximum slope of 35 %. Sources of water contamination like pesticides, fuel, oil, fertilizers, cleaning products and other compounds need to be controlled. Substrate contamination with heavy metals (e. g. cadmium, zinc, lead) and chemical residues are potential problems (Pacheco and Pohlan, 2005).

A soil check should be made prior to crop planting to establish whether there are troublesome pests in the proposed soil. Typical examples that may require control before planting are ants, cutworms, caterpillars, slugs, snails and wireworms. The endemic weed population should be assessed prior to cultivation with techniques that reduce herbicide use during crop growth and after transplanting it should be ensured that the herbs have minimal competition in the early stage of the crop. Techniques should be sought to break life cycles of any potential soil disease, for example by changing the soil moisture regime or inoculating antagonist organisms. This requires careful monitoring of herb production to avoid a monoculture situation with certain herbs (e.g. spearmint, sage, oregano).

Crop rotation is an important principle that improves soil fertility and reduces the risk of pest, weed and disease build up. The species used in this study belong to the *Lamiaceae*

family (basil, oregano, spearmint, sage) and rotations with species from the *Apiaceae* family (coriander, dill, parsley, chervil) are recommended. Oregano, spearmint, sage and basil require warm growing conditions and basic ground preparation before the rainy season. For Mexican conditions, the optimal season for planting herbs is after the coffee harvest, at the beginning of spring (Feb.-March).The soil should be given minimal preparation to maintain moisture, biological components and soil structure. A superficial till can be made before transplanting.

2.1.1 Propagation and establishment

Final plant density will vary according to the desired end use, method of cultivation and harvest. Leaf herbs can be grown by direct seeding, e.g. chervil, coriander, dill, and parsley. Others, such as spearmint, sage, tarragon, basil, chives, oregano and thyme, should be grown from vegetative propagates. In our case, basil, oregano and sage seedlings were produced in trays from seeds under controlled conditions and transplanted to the field. Spearmint was vegetative propagated.

Special attention should be given to seed quality, soil mixture and fertilizer placement in the trays. Some herbs, such as basil, sage and oregano, should be sown at or just below the soil surface. Others, with larger seeds, require dark conditions for germination and should be sown around 1-3 cm below soil surface. Most herbs should not be sown at soil temperatures below 10 ºC or establishment will be uneven. Basil and sage require higher soil temperatures. Regular applications of water may be necessary in the first month after transplanting to minimize plant losses. Thereafter, the transplants should be well established and further watering should relate to rate of growth and availability of resources.

The transplantation location should have adequate soil moisture and soil should be sufficiently deep to allow propagates to develop a decent root. Avoid transplanting under windy or dry conditions. Planting distances for basil and sage are between 50 and 70 cm, according to relief conditions. For spearmint and oregano increasing the planting density will help to establish a homogenous coverage and weed competition.

2.1.2 Agronomical management

2.1.2.1 Fertilization

A balanced nutrition for rapid crop establishment with strong root growth and early accumulation of green leaf tissue should be supplied after transplant. Before establishment of the experimental area, soil analyses were done for the six plots to be planted (three in each farm). Soils analyses are presented in annex 1 to 6. Fertilizer may be introduced during soil preparation by broadcast placement. In Mexico, 500 g of compost per plant (previous prepared in the farm) was incorporated at the moment of transplantation. As a complementary application, 50 g of a commercial fertilizer (NPK 46-0-8) was applied per plant and incorporated in the third week after establishment of the aromatic plants.

Special attention should be taken when fertilizing leaf herbs grown for harvesting by sequential "cuts" in order to replace the off-take with appropriate nutrients.

Under our experimental conditions, an application of a soluble fertilizer (NPK 17-17-17), at the commercial dosage (100 ml/plant), was used after every harvest in all herbs of the treatments.

2.1.2.2 Irrigation

As the annual variation in soil moisture is wide in tropical and subtropical regions, provision should generally be made for irrigating aromatic herbs. The form of irrigation should be determined by the growing system involved, as well as the species of herbs to be planted. As the leaves of these herbs are the product required, it is necessary to avoid flowering during the production period. Adequate water supply is critical at early stages of growth. Some herbs, such as basil, will bolt even if the seed or seedling are exposed to moisture stress, therefore, it is imperative that adequate soil moisture is available prior to sowing and throughout the leaf growth period. Generally, overhead irrigation systems are appropriate to irrigate annual herbs, although consideration has to be given to the possible problems arising from soil splash. For perennial herbs, drip irrigation is possibly a more appropriate method of irrigation, provided the installation costs can be justified. Good supply and management of water are imperative to sustainable economic herb production, in particular, timing of irrigation with respect to stage of plant growth, weather and time of day (Campos, 2006).

In the evaluated coffee production systems in the Sierra North of Puebla, no irrigation system was required. The area receives 2765 mm of rain during the year, with most of it falling between June, July and October.

2.1.2.3 Pest management

A wide range of pest problems can arise in leafy herb crops. These range from microscopic organisms to rabbits, hares and birds. Cutworms are a common problem, particularly in crops following grass.

Possibly the most obvious soil-borne pests are cutworms and wireworms, which were present in the preceding crop or weeds. They often manifest themselves by attacking quite large growing plants and gnawing at the root or stem base until the aerial parts collapse. Gaps are readily visible in the crop. These pests should normally have been monitored prior to cultivation of herbs (Bareño, 2006).

Ground beetles and other predators can help control some of these pests.

Problematic pests vary with the season and growing environment. Slugs, snails, trips, leafhoppers, whitefly and some caterpillars may be troublesome at any time of the year. Flea beetles and aphids may also become a problem early in the season.

2.1.2.4 Disease problems

The most common pathogenic fungus at the seedling stage includes *Rhizoctonia* spp., *Phytophthora* spp. and *Pythium* spp. (Elad, 1990). The most obvious symptoms are brown discoloration at stem bases, collapse of seedlings, causing gaps in the crop. Specialist attention is generally required to determine the exact causal organism and confirmation may be necessary before choosing an appropriate treatment. These disease organisms proliferate in wet soils and most commonly attack herbs when the soil temperature is cool and sub-optimal for good growth. Wet soil and cool temperatures lead to the rapid spread of damping off pathogens that move rapidly through soil moisture and attack weak or young growing plants. Foliar diseases can be split into those that occur under humid or under dry conditions. The group includes leaf spots, leaf blights and grey moulds; the latter includes powdery mildew, rusts and vascular wilts. There are common vascular wilts that arrest the water pathways within the plant. Typical examples are *Fusarium* spp. and *Verticillium* spp. *Verticillium* can arise in spearmint following another

perennial herb. *Fusarium* spp. has been found in basil. *Sclerotinia* spp. also attacks stem bases (Keinath, 1994; Gamliel *et al*., 1993).

Potential foliar diseases of the herbs studies include the following:

Septoria spp.: Symptoms arise as leaf spots most commonly in oregano, although they have been recorded in a number of other leafy herbs, including coriander.

Botrytis spp.: Commonly, Botrytis is the causal organism and can readily attack cut herbs under humid conditions. A wide range of leafy herbs suffers from this organism including woody labiates (sage, rosemary) and it is particularly common on delicate herbs (including spearmint and basil).

Oidium spp.: The powdery mildews can be widespread in dry hot weather, affecting a wide range of leaf herbs including parsley, dill, oregano and sage.

Puccinia spp.: Various species of rust fungi attack leaf herbs. They have been recorded on mint, tarragon, chives, oregano and thyme.

Control should be aimed at breaking the life cycle, as these pathogens can survive all year-round. No single treatment is likely to control rust. They provide an excellent example of the need for integrated management techniques (Guerrero, 2006).

2.1.2.5 Viruses and bacteria

Coriander has been recorded with bacterial blight and it can also succumb to bacterial wilt. Parsley can suffer from bacterial crown and leaf rots. Various viruses (e.g. carrot motley dwarf virus and celery mosaic virus) can distort *umbelliferous* leaf herbs. Spearmint can display symptoms of cucumber mosaic virus transmitted by aphids. Using an aphicide as a control of vectors or traps, may therefore be essential to reduce virus attacks (Elad, 1990; Mheen, 1993).

2.1.2.6 Physiological disorders

Symptoms of waterlogged conditions are generally first seen as purple or red colouring on leaf tips and margins, followed by either yellowing of the leaf blade or a spread of the red coloration. This can be a serious problem especially where herb crops are grown for the fresh market. As a number of aromatic herbs are not endemic to Latin American, ideal conditions for growth do not always occur, hence, stress-related disease and disorders can readily arise. Intensive monoculture also often leads to conditions conducive to rapid disease spread and actions to minimize these should be considered (De Muth, 1996).

2.1.2.7 Weed control

A number of weed species may occur in aromatic herb crops. Their influence is often greater as several herbs and weeds belong to the same weed plant families e.g. the *Apiaceae*. Furthermore if these weeds remain unchecked then flushes of different problematic weeds will occur at later stages during the growing season. Strategies need to be formulated to minimize these problems, as post crop emergence options are very limited and expensive (Bareño, 2006).

However, other control strategies should also be implemented wherever possible, including crop rotation, destroying diseased crop debris, substrate cleanliness and environmental control, particularly with regard to moisture and air movement.

2.1.3 Harvest and post-harvest management of herbs

Generally, leaf development is the main criterion that determines harvest time. The crop plants should be true to type, free of pests and diseases, have a good colour and be weed free, of optimum size and turgid. It is difficult to determine all the variations that may be desired in the final product, however, some common considerations ensure that the desired stage is not passed before harvest e.g. some leaves can change within days from a rosette to a highly divided leaf (“carrot leaf”) with an accompanying change in flavour that may negate the marketable value of the crop. With other crops, the petiole may become longer than desired if the correct growth stage is not correctly identified at harvest (Pacheco and Pohlan, 2006).

The most frequent problem is a change from vegetative to flowering development. This usually occurs at certain defined times of the year or in response to stress factors, as in basil. Most leaf herbs have a known flowering period which is more or less constant year after year. To avoid unnecessary losses, observation and crop planning may require proactive steps to be taken. By simultaneously growing susceptible herbs under various environment conditions, you minimize the risk of loss from flowering influences. The selection of cultivars with different flowering periods can assist continuity of supply.

Therefore, harvest procedures have to be carefully planned and workers well trained to ensure a sustainable crop (Villamizar, 2001b). By diligent attention to post-harvest procedures, a great deal of pest, weed or disease control can be incorporated. When herb crops are maintained for more than one cut, it is often prudent to apply certain maintenance treatments immediately after cutting e.g. an application of mancoceb (Manganese ethylene bisdithiocarbamte, 80 percent active ingredient) or another fungicide prevents the development of bacteria and fungus in the exposed tissue. Application of fertilizers may also be opportune to replace some of the lost nutrients.

Most cut herbs require an ambient storage temperature of 0-4 ºC in a high humidity environment. Once stabilized at these temperatures, cut herbs may be processed further. Optimum post-harvest temperatures for a maximum shelf life of herbs should be between 6 to 10 ºC and they should be well watered before dispatch. If herbs are not cooled sufficiently under the correct conditions after harvest, a number of different reactions can occur. Some herbs display yellow discoloration of leaves (e.g. basil and dill) or exude fluids which will rot the plant tissue (e.g. chives, oregano and mint) (Pacheco *et al*., 2004).

Herbs can be packaged in bags designed to minimize water loss. When herbs are packaged this way, it is particularly important to maintain constant temperatures, to reduce condensation inside the bag and the consequent risk of fungal or bacterial growth. The bags may be partially ventilated with perforations, or may be constructed of a polymer that is partially permeable to water vapour. The relative humidly in the packing area, cold rooms and transport vehicles should be maintained at a high level (>95%) where practical.

Ethylene gas is another factor which limits the shelf life of leafy tissues. Ethylene causes yellowing of leaves and an increased rate of deterioration. It is possible to routinely find

one to three ppm ethylene in the environment surrounding fruits and vegetables during commercial handling. Young growing herb tissue responds to ethylene (5 ppm), whereas little effect was observed in mature herb cuttings. In addition, holding the herbs at the recommended temperatures also greatly reduces their ability to respond to ethylene in the environment (Cantwell and Reid, 1993). Careful handling to avoid physical injury to the leafy tissue of the fresh herbs is also important. Rigid clear plastic containers such as those sometimes used for sprouts may be used for soft herbs. "Pillow packs" (plastic bags which are partially inflated when sealed) may be an alternative packaging technique

2.1.3.1 Modified atmosphere packaging (MA)

The quest of hygienic, fresh and high-quality food products has lead the food sector to develop the packing system for food products under modified atmospheres packaging (MA)[2].The packing method implies the elimination of the air from the inside of a container and its substitution for a gas or mixture of gases, depending of the type of product to be packed (Church and Parsons, 1995; Phillips, 1996).

The gaseous atmosphere within a package changes continuously during the storage period under the influence of distinct factors such as respiration of the product, biochemical changes, and the slow diffusion of gases through the package walls.

In the modified atmosphere technique, four basic components are important: the container used the mixture of gases, the materials of the container and the packing equipment.

The normal composition of gases utilize under MA is: 21 % oxygen, 78 % nitrogen and less than 0.1 % of carbon dioxide. Oxygen (O_2) is the most important gas involved in MA, used principally by aerobic micro-organisms which cause decomposition, and necessary in some enzymatic reactions in foodstuff. For these reasons, in modified atmospheres, O_2 is reduced to very low levels (Parry, 1993). Exceptions are made when it is necessary to maintain or achieve desired qualities of certain foodstuffs, such as for respiration of fruits

[2]Additional information on the MA system can be obtained from a variety of sources including the following web-sites

www.postharvest.com.au
www.modifiedatmospherepackaging.com
www.davisfreshtech.com/articles_map.html

and vegetables, the retention of colour in red meat, or to avoid anaerobic conditions when whitefish is stored.

Carbon dioxide (CO_2) produces an inhibitory effect on bacterial growth, particularly against decomposing gram negative aerobic bacteria, such like *Pseudomonas* spp., that cause a loss of colour and bad odours in meat and fishes. Others, like acid lactic bacteria and yeasts, grow upon presence of carbon dioxide which has effect on them. Nitrogen (N_2) is an inert gas, with low solubility both in water and greases, utilized fundamentally in modified atmospheres to displace the O_2, and well as for preventing the staleness in dried fruit (Kays, 1999).

The advantages of modified atmosphere packaging are:

- Lengthened storage time, which permits transportation over larger distances and replacement of sold items on shelves with frequency.
- Retailer's reduction of level refuses.
- Better presentation of the final product.
- Hygienic storage and transportation of containers, since these are closed and the products do not smell or drip.
- Less or no need of chemical preservatives.

The inconveniences of modified atmosphere packaging are:

- Investment in machinery of bottle carbonated.
- Cost of gases and production materials.
- Investments in analytical teams to guarantee proper mixing of suitable gases.
- Expenses in systems to assure the quality of packages, in order to avoid distribution with perforations.
- Need of more storing space in trucks and other transportation vehicles.

2.1.3.2 Methods of atmosphere modification for packing herbs

There are two main means of packaging: vacuum package and gaseous package.

Vacuum packaging

It is the modified atmosphere package method first commercially developed and it is still broadly used. It is not indicated for soft or bakery products because the vacuum process produces an irreversible deformation of the product. In the case of aromatic herbs, compression and deformation does not represent a problem that prevents product commercialization.

Gaseous packaging

It can be obtained either by generating the atmosphere inside the container, or by mechanically replacing the air with a gas or gas mixture. At the same time each technique shows two methodological variations. The former method, generating the atmosphere, presents a passive variation, as in the case of fruits and vegetables, and an active variation that induces atmosphere modifications using, for example, oxygen absorbents.

On this study both methodologies were compared and evaluated looking for an alternative packing method for herbs that prevent losses in shelf life and reduce commercial opportunities.

2.2. Allelopathic potential of herbs

Chemical signals are very common in many organisms. Both plants and animals use odours and scents as communication mechanisms. The term allelopathy (from the Greek Allelon = one to another and Pathos= to suffer) was coined by Molisch in 1937 (Rice, 1987) to refer to reciprocal suffering of two organisms. Allelopathy is commonly defined as any direct or indirect effect, stimulatory or inhibitory, mediated by a chemical compound, released into the environment by a given plant or micro-organism (Rice, 1984). These chemical compounds may be produced directly by living plants or indirectly through the products of plant decomposition. Allelochemicals can be released into the environment through a variety of mechanisms: volatilization from leaves, exudation from roots and leaching from fallen leaves and plants litter (Putnam and Tang 1986, Putnam and Weston, 1989,; Rizvi *et al*., 1999).

In every allelopathic relation there exists a plant (donor) that frees chemical compounds into the environment (atmosphere or rhizosphere) through a specific way (volatilization, leaching, decomposition of residues and root exudation) which are then absorbed by another plant (receptor), causing a damaging or a beneficial effect (Chou, 1986).

According to Putnam and Weston (1989), chemicals with allelopathic potential are present (commonly in their conjugated form) in almost all plants and in many tissues like leaves, stems, flowers, fruits and seeds. In nature, the plants are exposed to biotic and abiotic factors that cause the development and accumulation of secondary metabolites, which then cause specific effects on other plants or animals.

The principal studies on allelopathy in Latin America have been carried out in natural ecosystems, mainly in the secondary communities that replace the rain forest when it is destroyed for agricultural practices (Anaya *et al*., 1992). As reported by Ramos (1991), some species of these communities produce one or more substances (allelochemicals), mainly in the leaves or through the decomposition of their organic matter, that can inhibit plant growth or otherwise influence surrounding plants. The production of these compounds in tropical zones, particularly if they are continuously released into the environment, may contribute to the elimination of secondary or competitive species and to the selection of those which are well established in the community. Müller, (1970) initiates a long series of research activities in the field of allelopathy induced by aromatic plants with the observation of striking patterns of vegetation in and around patches of *Salvia* and *Artemisia* spp. in the costal sage communities of California, USA. These patterns were considered to be the outcome of inhibitory effects exerted by the volatile oils emitted by *Salvia* and *Artemisia* plants. These compounds are synthesized and stored in a special structure called gland, which is located in different parts of the plant, such as leaves, flowers, fruits, seeds, barks and roots. Generally, herbs products (chemicals) can be divided in two groups: primary metabolites, such as sugars and nucleic acids, produced by every plant, and secondary metabolites, which include all other chemicals that a plant may produce. Secondary metabolites belong to different chemical classes such as phenolic acids, tannins, flavonoids, terpenoids, alkaloids, steroids and quinons (Chou and Waller, 1980b; Einhelling and Leather, 1988; Duke *et al*., 2000; Phippen and Simon, 2000).

Due to the essential oils rich in phenolic complexes and a wide array of other natural products, including polyphenols such as flavonoids and anthocyanins, produced by aromatic plants, allelopathic effects caused by these plants have long been studied (Phippen and Simon, 1998).

Allelopathy has gained much attention in the field of agro-forestry and weed science. Appleton (2000) suggests that allelopathy enhances tree survival and reproduction, and that plants producing allelochemicals can be used in production as cover crops to control weeds. For example, the black walnuts (*Juglans nigra*) accumulate hydrojuglone (in leaves, stems, fruits and roots) which when exposed to air, oxidizes to toxic juglone. The juglone can volatilize from the leaves, or leach by rain drops. When in contact with neighbouring plants or weeds, it causes them to yellow, wilt, and dies. It is not known whether host plants could employ surface flavonoids in aerial chemical warfare, to directly suppress the growth of weeds, or cause wilting or death of weeds or neighbouring plants.

However, there is evidence that exudates and/or surface flavonoids could have allelopathic potentials when secreted into the soils (Bais *et al.,* 2002; Stermitz *et al*., 2003; Weir *et al*., 2003). Vivanco and co-workers (Bais *et al*., 2002) isolated and showed that (−)-catechin is a root-secreted phytotoxin that undoubtedly contributes to knapweed's (*Centaurea maculosa*) invasive behaviour in the rhizosphere. Similarly, the surface flavonoids of *Cistus ladanifer* (*Cistaceae*), apigenin-4'-methyl ether and kaemperol-3,7-dimethyl inhibited the development of the seedlings of *Rumex crispus* (Chaves *et al*., 2001). The flavonoids were also detected in the soils associated with *Cistus ladanifer*, confirming their allelopathic potentials. In other studies has been demonstrated that secondary metabolites may serve as chemical defence compounds against herbivores and predators, microbes, viruses or competing plants. Further research has shown that secondary metabolites function as signal molecules in plant-plant, plant-animal and plant-microbe interactions. (Johns, 1985; Holopainen, 2004; Tholl *et al*. 2006.

According to Glass (1974), a group of phenolics, namely benzoic and cinnamic acids, present in aromatic plants are potent inhibitors of K and P absorption. The effect is readily reversible and the inhibitory capacity of the benzoic acids is strongly correlated with the

lipid solubility of the cell membrane, modifying its permeability and thereby increasing the rate of exchange (Douglas, 1992). These initial actions on cell membranes result in unspecific permeability changes that alter ion fluxes and hydraulic conductivity of roots. Membrane perturbations are followed by a cascade of secondary physiological effects that include alterations in ion balance, plant-water relationships, stomata function, and rates of photosynthesis and respiration (Einhellig, 2004). As seen some allelopathic effects has been elucidated and their mode of action elucidated, however, large number of reported cases the allelopathic compounds and their mode of action are not yet understood.

Most of the research projects in allelopathy have been designed in such a way that only the inhibitory results are considered significant. The scientists have been very aware, however, of the significance of the chemical stimulation of growth and development of plants by other plants volatiles or interactions. Instances of allelopathic stimulation of coffee plants by intercropping herbs are the result of previous experiences. This speculations need to be tested and the potential interactions identified.

2.2.1. Growth stimulation capacity

Some of the requirements of intensive agriculture make the use of plant growth stimulation products necessary for continuous production. High residues of these products, caused by practices such as the excessive use of nitrogen fertilizers (urea) or hormonal stimulants can have a constraining effect on the trade and export of some vegetables.

Plant growth and physiological properties can differ markedly under the influences of neighbour plants. Although most reports ascribe negative allelopathic effects to aromatic plants and an inhibitory influence to surrounding plants (Rice, 1984), some researchers mention positive influences on the growth of some plants and micro-organisms, in the presence of chemical stimuli. Volatile terpenes and essential oils have been reported as regulators of germination and growth of other species in several ecosystems (Curl and Truelove, 1986).Another report from Rice (1987) indicates the growth increase of radish and downy brome grass by *Glechoma hederacea* (ground-ivy). Decaying ground-ivy leaves (2g/kg of soil) stimulated growth of downy brome shoots by as much as 770 % and

radish shoots by as much as 1064 %. Downy brome root growth was stimulated up to 251 % and radish root growth up to 1354%.

Regulation of the concentrations of hormones, such as auxins and gibberellins, is also important for normal plant cell growth and morphogenesis. Some flavonoid aglycones act to inhibit polar auxin transport, leading to a disturbance in normal auxin levels and resulting in the induction of lateral roots and the suppression of ageotropic growth (Brunn *et al.,* 1992). Some other authors also report an auxin-protecting activity of some phenolic compounds by inhibiting the peroxidase- and oxidasecatalyzed oxidation of auxin (Mato *et al*., 1994; Cvikrova *et al.,* 1996).As reported by Peer *et al* (2001), phenolic compounds, principally flavonoids, have a possible role in the regulation of auxin retention. Flavonoids comprise a diverse group of phenolic compounds that serve a variety of ecological and physiological function in plants, and are present in aromatic plants, principally in basil, sage, oregano and rosemary (Stafford, 1990; Dabeaujon *et al*., 2000). Murphy *et al*. (2000) demonstrated a tissue-specific localization of the flavonoids kaempferol, querceritin and naringenin chalcone in the hypocotyls-root and in the distal elongation region of the root in *Arabidopsis* seedlings in correlation to auxin retention. Peer *et al*. (2001) mention on their work that flavonoids co-localize spatially and temporally with regions of auxin accumulation. They also found that the localization of aglycone flavonoids in areas of organ transition and maturation suggests that flavonoids influence developmental processes in through controlling the distribution of auxin in tissues.

In this study, the potential stimulation of coffee plant growth due to exposure to volatile essential oils (VEOs) emitted from intercropped aromatic plants was investigated. Previous studies in Chiapas, Mexico in 2003 revealed that the growth of coffee plants was stimulated primarily by intercropped chives and sage but mechanisms of action are unknown. To determine whether or not the VEOs from herbs are involved in the stimulation process and if factors such as concentration, herb species and time of application are of importance for the potential effect, experiments with different concentrations of VEOs from spearmint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), sage (*Salvia officinalis* L.), and oregano (*Origanum vulgare* L.) need to be evaluated as growth stimulants.

2.2.2. Antioxidant properties

Plant antioxidants and their role in the plant defence system have received increasing attention within the last decades (Larson, 1988; Alscher and Hess, 1993; Smirnoff, 1995). The importance of aromatic and medicinal plants depends on the high content of chemical compounds exhibiting antioxidant properties. Their main constituents, (poly) phenolic substances are a class of higher plant secondary metabolites. They tend to be water soluble, frequently occur as glycosides and are usually located in cell vacuoles (Harborne, 1998). Polyphenols are antioxidants with redox properties. The common compounds present in herbs like cinnamic and benzoic acids, coumarines, tannins and flavonoids, have redox properties that allow them to act as reducing agents, hydrogen donators and single oxygen quenchers (Einhelling and Leather, 1988). Another complementary report (Proestos *et al*., 2005) shows evidence of the antioxidant capacity of herbs of the *Labiatae* family in relation with the total phenolic content expressed as PF Values (Table 1).

Table 1: Total phenolic content in herb extracts expressed as protection factor values (antioxidant capacity)

Name of Herb	Part examined	Drying Method[1]	Total Phenolics[2]	PF[3] (ground material)	PF (methanol extracts)
Salvia officinalis	Leaves	air	13.6 +/- 0.4	1.4	1.2
Thymus vulgaris	Herb	air	19.2 +/- 0.3	4.7	4.1
Mentha viridis	Leaves	air	16.5 +/- 0.3	1.4	1.3
Rosmarinus officinalis	Leaves	air	21 +/- 0.5	5.1	4.5
Origanum vulgare	Herb	air	16.9 +/- 0.3	2.1	1.8
Lavandula vera	Flower	air	7.3 +/- 0.2	1.3	1.2
Mentha piperita	Leaves	air	8.4 +/- 0.1	1.6	1.4
Melissa officinalis	Leaves	air	17 +/- 0.3	1.4	1.2
Ocynum basilicum	Leaves	air	3,1+/-0,2	1,4	1,1

Source: Proestos *et al.*, 2005

1 air dying f/v, freeze vacuum, i.e. lyophilisation

2 mg of Gallic acid /g of dry sample

3 PF: protection factor CV (%) =3.4 n=3

There is a great incentive to discover effective and economically feasible stimulant properties that could be introduced in production in environmental friendly production

systems. This study of the physiological role of natural products in biochemical interactions among coffee plants and some herbs can supply new active compounds and/or models for new mechanisms of action.

2.3. Caffeine accumulation and potential uptake through intercropped herbs

The traditional coffee production systems in Latin America are a consequence of deforestation with agricultural objectives and are characterized by effects from the shrub layer (coffee plants) into the herbaceous layer (Anaya *et al*., 1999). The purine alkaloid caffeine is a biologically active compound found in members of the *Rubiaceae* family that contribute to allelopathic and auto-toxic effects appearing in coffee plantations (Rizvi and Rizvi, 1987; Suzuki and Waller, 1987; Anaya *et al*., 2002).

Friedman and Waller (1983a) noted that around old coffee trees, caffeine was released from the tree's own litter and, over the years, accumulated in the vicinity of roots and leached out of the seeds into aqueous media, suggesting storage in a soluble form in the soil. Since most coffee roots develop in the upper soil layer, immediately under the tree's own litter, auto-toxicity can be manifested in soils of long establishment of monoculture regarding one reason for "low production phenomena" (Rizvi and Rizvi, 1987; Suzuki and Waller, 1987).

Coffee plantations are managed in different ways, ranging from intensive cultivation to maintenance as natural forest-like ecosystems, but no attention has been given to allelopathic effects that are produced by secondary metabolites such as caffeine, present in *Coffea arabica* (Chou and Waller, 1980a).

Frischknecht *et al*. (1986) concluded that caffeine might be an effective defence mechanism in young coffee plant leaves. The high amount of caffeine content in young buds and leaflets indicates that its purpose is to defend the plant from predators who would feed on these nutrient-rich organs. Caffeine was also shown to be an efficient repellent and toxicant against slugs and snails (Hollingsworth *et al*., 2002). Anaya *et al*. (1992) report that lixiviated coffee leaves and roots, as well as extracts from soil contain (among other compounds) caffeine and methyl esters of myristic through docosanoic acids, which have allopathic activity against predators.

C. arabica not only requires a method to protect its leaves, but a method to compete for essential nutrients in a stressful environment. According to estimates, the annual amount of leaf-litter produced by old coffee trees, plus about 10 % of fallen fruits is between 150-200 g dry matter/m^2/year (Epifanio, 1981). This organic matter releases 1-2 g caffeine/m^2/year in addition to other coffee constituents. Douglas (1992) reports that caffeine inhibited shoot elongation by half after 6 days of growth, and inhibited root elongation by 90% compared to control in seedlings of rice plants (*Oryza sativa* L. cv. *Lemont*) germinated in water. Friedman and Waller (1983b) showed that caffeine inhibits mitosis in lettuce (*Lactuca sativa* L.) roots. These authors also concluded that caffeine is a powerful allelochemical that inhibits mitosis in the roots of many plants and reduces their access to nutrients and water.

Caffeine accumulated in coffee could be a restriction factor for an intercropped system and complementary processes for detoxifying the soil are desirable. Waller *et al*. (1986) demonstrated the uptake of exogenously applied caffeine by coffee rootlets (*Coffea arabica* L. cv. *Bourbon*) and its translocation mainly to the shoots. However, it was not investigated, whether caffeine was just accumulated or was subsequently conjugated to primary metabolites such as sugars. Detoxifying reactions, relatively widespread in the plant kingdom, enable plants to overcome inhibitory effects of natural or artificial phytotoxins and help them to overcome allelopathic attacks by neighbouring plants and to grow in adverse environments (Schulz and Friebe, 1999).

Previous studies show evidence of caffeine uptake by aromatic plants and its translocation mainly to the roots, diminishing caffeine content of coffee soils (Pacheco *et al*., 2006). The same study mentioned changes in morphological characteristics in thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) and a decrease of active UV compounds in sage (*Salvia officinalis* L.) and oregano (*Origanum vulgare* L.) upon caffeine uptake. This phenomenon provides strong evidence of a possible reaction of the plants to phytotoxic compounds, and a change in the chemical characteristics of the essential oils. According to Anaya *et al*. (1999), plants can avoid phytotoxicity by storing toxins (e.g. caffeine, theobromine) into their vacuoles or hairy glands with subsequent changes in the physical characteristics.

Caffeine in nature is metabolized by micro-organisms which are adapted to the presence of this alkaloid, and may use it as a carbon resource. Nevertheless, while this natural detoxification process decreases the caffeine content in the soil, it is not enough to avoid accumulation of the allelochemical to toxic levels in coffee plantations. If some aromatic species can take-up caffeine and help in its degradation, they may help to diminish the problem and reduce the "low production" phenomena.

Aromatic species have an important role on the coffee production system as intercrops, but more experimental evidence and stronger argumentation is needed to validate the hypothesis that herbs can help detoxify caffeine-containing soils under coffee production.

2.4. Cup quality in coffee

The main focus of coffee research and technology transfer has been on increasing production volumes. The downward trend of prices for commodity coffee in particular during the most recent coffee crisis and its related socio-economic and ecological consequences has shifted increased attention to high quality coffees. To obtain the often very lucrative premiums for specialty-grade parchment beans farmers and their associations must be able to enter markets that have very stringent food quality and food safety requirements (Läderach *et al*., 2006).

Several studies have attempted to establish a relationship between the chemical composition of green coffee beans with beverage quality, seeking substances or precursors which may underlie the acceptance or rejection of the beverage (Clifford, 1985). Proteins, amino acids, carbohydrates and phenolic compounds have been indicated as important compounds for the development of good coffee beverage (Clifford, 1985; 1997; Rogers *et al*., 1999a, b; Shimizu and Mazzafera, 2000; Selmar *et al*. 2001; Montavon *et al*., 2003a, b). The action of polyphenoloxidase (Amorim and Amorim, 1977) and proteases upon some of these constituents has also been suggested to play a role in determining the quality of coffee although the importance of polyphenoloxidase in this respect has been questioned (Mazzafera, 1991; 1999; Mazzafera *et al*., 2002).

The concentrations and the mechanisms of action for the compounds mentioned above and how they affect the beverage quality remain unknown (Montavon *et al*., 2003a). However,

in addition to the genetic background (Carvalho, 1988; Scholz *et al*., 2001) and the harvesting and post-harvesting procedures (Vincent, 1985) the production of good quality coffee beans in specific areas, characterized by their climatic conditions clearly shows that the climate is an important factor in determining the quality of the coffee beverage.

Coffee cup quality depends on environmental and genetic factors, as well as on agronomic production and post-harvest management. The dependency of cup quality on these factors has been described in a few very recent studies (Alarcon-Mendez *et al*., 1996; Avelino *et al*., 2005; Decazy *et al*., 2003; Muschler, 2001; Vaast *et al*., 2004) but no attention has been given to plant-plant interaction and potential allelopathic effects of intercropped cultures in coffee production systems and its effect on cup quality of coffee.

Coffee quality is to be understood as a combination of physical characteristics (bean size and weight), chemical characteristics (caffeine, trigonelline, sucrose and chlorogenic content), as well as cup quality, expressed as aroma, body, acidity, bitterness, amongst others. The chemical composition is determined by a complex interaction of agricultural factors, roasting, blending, and brewing (NECC, 2002; Decazy *et al*., 2003; Franca *et al*., 2005; Silva *et al*., 2005). The main constituents of coffee have been known for over half a century. In order of abundance, typical values for the water soluble constituents are: phenolic polymers (8%), polysaccharides (6 %), chlorogenic acids (4 %), minerals (3 %), water (2 %), caffeine (1 %), organic acids (0.5 %), sugars (0.3 %), lipids (0.2 %) and aroma (0.1 %) (ICS, 2001; Rubayiza and Meurens, 2005). The criteria commonly used to evaluate the quality of coffee beans include bean size, colour, shape, processing method, flavour or cup quality, the presence of defects and others. Among these criteria, flavour is the most important criterion for coffee quality evaluation and most employed worldwide in coffee trading (Franca *et al*., 2005; Farah *et al*., unpublished data).

Coffee flavour is developed during the roasting process from aroma precursors present in green beans (Ky *et al*., 2000). It is the product of a complex chain of chemical transformations. The green bean has only a faint odour that is not at all reminiscent of coffee aroma, but it contains all of the necessary precursors to generate the unique coffee flavour during roasting. The chemistry of flavour development during coffee roasting is highly complex (Farah *et al*., unpublished data). Roasting is a pyrolytic (heat-driven)

process that greatly increases the chemical complexity of coffee. Although the roasting process appears to be simple in terms of processing conditions, it is quite complex from a chemistry point of view, since hundreds of chemical reactions (degradation of proteins, polysaccharides, trigonelline and chlorogenic acids) take place simultaneously (De Maria *et al*., 1996). During roasting, coffee bean volume increases by half or more; but bean mass decreases by a fifth. Moreover, the number of volatile molecular species responsible for aroma formation increases from some 250 in green coffee to more than 800 in roasted beans (Illy, 2002).

Sucrose is the principal sugar in coffee, and it will act as an aroma precursor, originating several substances (furans, aldehydes, carboxyl acids, etc.) that will affect both flavour and aroma of the beverage. The higher the sucrose content in green beans, the more intense coffee cup flavour. Trigonelline is 100 % soluble in water and therefore will end up in the cup. It is probably the most significant constituent contributing to excessive bitterness (Ky *et al*., 2001). Therefore, enhancing coffee quality would imply increasing sucrose and trigonelline contents while decreasing CGA and caffeine contents (Ky *et al*., 2001). The levels and biochemical status of these precursors may vary in relation to local environmental conditions.

3 METHODOLOGY

3.1 Laboratory bioassays

3.1.1 Caffeine uptake and accumulation by herbs

To ascertain the caffeine is uptake by aromatic plants, and to look for the physiological effects and possible destabilization of secondary elements, aromatic plants were exposed to a concentration of caffeine under controlled conditions. Four species of aromatic plants spearmint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), sage (*Salvia officinalis* L.), and oregano (*Origanum vulgare* L.) were germinated from commercial seeds (Sperli seeds) in 20 x 40 x 5 cm trays under greenhouse conditions using a mixture of 1 part (200 g) of potting soil and 2 parts (400 g) of peat moss. The trays were watered *ad libitum* and maintained at continuous temperature (between 25-30 °C).

Six weeks old seedlings were transferred in glass beakers (500 ml) with their root system dipping into 500 µM caffeine solution in 250 ml incubation medium, prepared as described in Schulz and Wieland (1999), but with caffeine instead of benzoxazolinone. Controls were incubated without caffeine. Exposure times were 24, 48 and 72 hours. The beakers were closed with parafilm (Pechiney PM996) to prevent evaporation and changes in the concentration of the medium and artificial aerated (Figure 4).

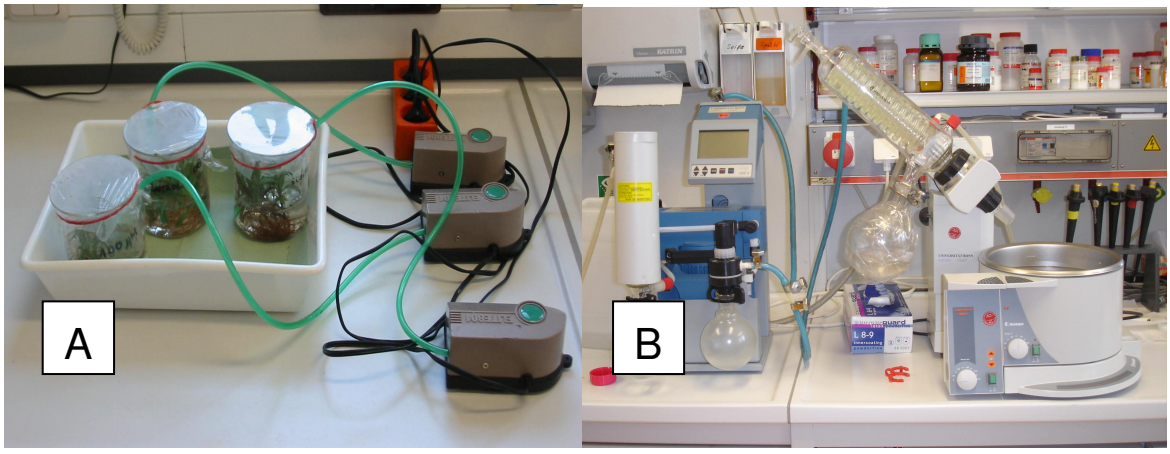

Figure 4: A: Experimental conditions for caffeine uptake bioassays.
B: Experimental conditions for vacuum evaporation of release samples.
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To estimate the uptake of caffeine in the aromatic plants, aerial parts (shoot and leaves) and roots were harvest separately. The plant material was weighted and samples from 2 g were mortared and homogenized with 50 % Methanol (v/w 2/1) and quartz sand. The mixture was filtered through Miracloth and centrifuged at 4 ºC and 14000 g_n for 20 min.

The supernatants were collected. Samples of the medium after uptake were analyzed to estimate the remaining caffeine content. Identity and quantity of the compounds present on different plants tissues was established by methanolic extraction and High Performance Liquid Chromatography (HPLC) analysis.

3.1.2 Release of caffeine by aromatic plants

For the determination of a possible caffeine release after uptake, six-weeks-old plants after 48 h incubation with 500 µM caffeine in 250 mL incubation medium were analyzed. Four plants per specie were placed in 500 mL beakers with 200 mL aerated tap water. After 24, 48 and 72 h the total volume of water was replaced with fresh water. The water containing released products was evaporated under vacuum (Figure 4).

The residue was dissolved in 1 ml 70 % methanol and the solution centrifuged at 4 ºC and 14000 g_n for 15 min. The supernatant was used for HPLC analyses.

To analyze if there are still some residues of the alkaloid in the plant material after 72 h, the roots, shoot and leaves, were separated and samples of 1 g homogenized with 50 % Methanol (v/w 2/1) and quartz sand. The mixture was filtered through Miracloth (Calbiochem) and centrifuged for 20 min and 14000 g_n. The supernatant as used for HPLC analyses.

HPLC analysis

HPLC analyses were performed with a Beckman 126 chromatograph equipped with a diode array detector 168 and an ultrasphere ODS RP 18 column. Compounds were eluted with the following gradient: 0-20 % B in A for 1 min - 20- B in 100% A in 20 min - 20 min 100% B, using 0.1% TFA (Sigma) in H2O as eluent A and methanol (Baker) as eluent B at a flow rate of 1 ml/min. The detected wavelengths were 280 and 227 nm (Knop *et al*., 2007). Absorbed caffeine was identified by co-chromatography with the synthetic caffeine (Roth) as the reference substance and by UV- scan (max = 210, 273).

3.1.3 Influence of volatiles from sage, spearmint, oregano and basil on coffee leaf stomata aperture

Four weeks single plants of the aromatic species were placed into glass exsiccators together with a sixth month old *C. arabica* (20 cm high) plant. Controls were set up without exposure to aromatic plants. Air circulation was guaranteed by small ventilators inside of the exsiccators and exchange with the environment outside by two small openings, as described in Schulz *et al*. (unpublished data). The experiments were placed in a phytotron and watered *ad libitum* (Figure 5).

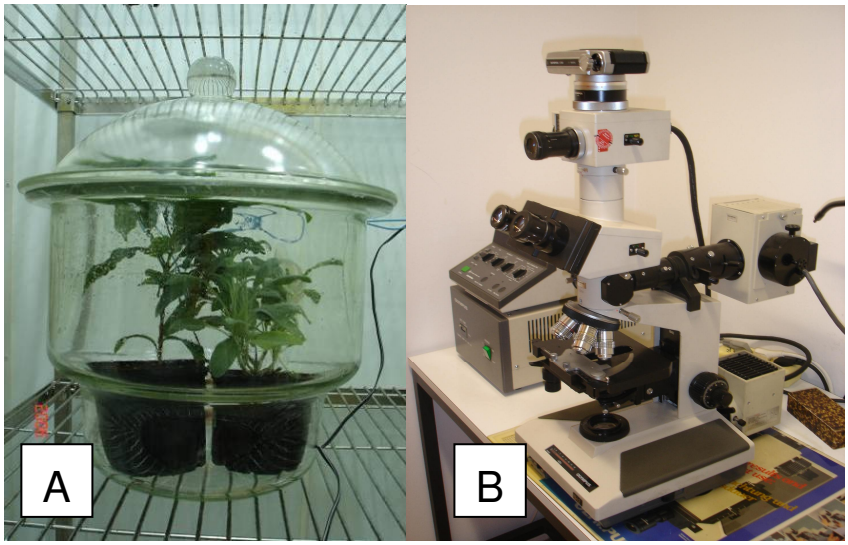

Figure 5: **A:** Experimental conditions of incubation *Coffea arabica* with sage (*Salvia officinalis* L.) under air controlled conditions in glass exsiccators.
B: Microscope Olympus BH2 with photographic equipment PM10 AD used for stomata observation and measurement of aperture.
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Observations of the stomata aperture of the leaves (n=50) were noted and the means statistically analysed with the SPSS 12.0 software (One-way ANOVA, Tukey's test at 95 % confidence).

Leaves of the coffee form the lower, media and upper part of the plant were harvested after 24, 48 and 72 h of exposure to volatiles of aromatic plants. The leaves under surfaces were covered with transparent tape; the upper parts of the leaves were stripped using a scalpel blade and residues of the mesophyll carefully removed. The tapes with the epidermis of the under surfaces were adhered to a slide and immediately used for microscopically

measurements of stomata aperture (Olympus BH2 with photographic equipment PM10 AD). A 60x ocular SWHK was used for calibration (Figure 5).

3.2 Field Research

3.2.1 Interaction of aromatic plants in coffee production systems

The experimental areas were located in the rural area of Xicotepec de Juárez, coffee production area of Puebla State, Mexico, in two different conditions farms, the first called "La Providencia", located at 20° 16' 295" N and 97° 50' 456" W with an altitude of 900 m above sea level and the second "La Orquidea" located at 20° 16', 887" N and 97° 45' 600" W with an altitude of 550 m above sea level. The experiments were establish and evaluated between November 2004 and December 2005 (Figure 6).

Source: www.pickatrail.com

Figure 6: Localization of experimental area in Mexico.
Puebla State produces 36% of the national production of *C. arabica* in Mexico (Gómez, 1998). The municipality of Xicotepec de Juarez is one of the mostly representative from the North Sierra of Puebla State.

Ecological conditions

Annual mean temperature varies strongly according to the altitude between 18-24 °C for the experimental area (between 550 and 900 m.a.s.l.). Annual mean precipitation is 2765 mm and 80% of the rains precipitate in the period between June and October.

The meteorological data for the region evaluated is presented in the table 2.

Table 2: Meteorological information for the experimental region, Puebla, Mexico (2005)

January			February			March			April			May			June		
Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.
18,6	11,5	38,1	18,8	12,1	63,9	22,2	13,5	33,4	24,9	15,9	65,5	24,6	17	137	26,3	18,8	466
July			August			September			October			November			December		
Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.	Tmax	Tmin	Prec.
24,5	17,1	373	25,3	17,5	508	23,3	17,1	621	23,6	16,8	392	21,6	13,7	30,8	17,6	10	36

T in Cº

Prec. in mm

Source: Meteorological Station Orquidea farm, Xicotepec de Juárez, Puebla, Mexico.

The region geologically is very uniform, is constituted of Granite of the Paleozoic. Because their soils characteristics, the predominant are Acrisol, associates with Andosols and Regosoles. The organic carbon content is low. The soils have a good to very high organic matter content. For temperate soils, the equilibrium C/N ratio has often been taken as 10/1 with somewhat lowers values in soils with higher temperatures and more microbiological activity. The N content is low to medium. The effects of low pH (< 6.0) on nitrogen availability are particularly market, in that microbiological activity is considerably reduced and available N is consequently very low. There is enough iron at all sites, but high phosphate levels can inhibit the uptake of iron. The zinc availability is greatly affected by soil pH. Deficiency symptoms rarely occur in acid soils unless native reserves are very low. Phosphorus can also induce zinc deficiencies, when available phosphorus levels in the soil are high or when high levels of phosphorus levels are added.

The strong interaction which is generally held to occur between copper and soil organic matter does not affect copper availability to plants, although it does influence the concentration of copper in soil solutions. Toxic levels of manganese are most common in acidic soils with pH values of about 5.5 or less, but this is not the matter in this sites. Soil analyses of the plots from both ecological conditions are presented in annex 1-6.

The climate of the area is classified as A (C) m (w): semi hot, humid with abundant rains in summer according to Köppen classification (Table 3).

Table 3: Climatologic conditions for coffee regions in Puebla, Mexico according to Köppen classification.

Type of climate*	Annual precipitation (mm)			Medium annual temperature °C			Evapotrans-piration (mm/year)
	Max.	Med.	Min.	Jan. (Min.)	Med.	April (Max.)	
Aw1 (w)	1518	1143	784	27,3	28,1	28,8	1653,0
Aw2 (w)	1929	1334	850	27,0	28,0	29,2	1587,6
Am	2745	2085	1311	26,5	27,2	28,5	1548,8
Am (w)	4087	3269	2387	26,6	27,5	28,8	1507,7
Am	3101	2395	1775	27,1	27,9	29,3	1638,8
A(C) m (w)	5254	3914	2884	23,4	23,9	24,6	1133,2
C(m)(w)	-	-	-	-	-	-	-
Cw2 (w)	1654	1255	832	20,0	21,2	22,2	1270,4

Note: C (m)(w): moderate humid with abundant rains in summer
Cw2 (w): moderate sub humid with rains in summer
A(C) m (w): semi hot, humid with abundant rains in summer
Am (w): hot humid with abundant rains in summer
Aw2 (w): hot sub humid with rains in summer
Aw1 (w): hot sub humid with rains in summer
Am: hot humid with abundant rains

The farms cover a surface of 100 ha^{-1} (Providencia farm) and 80 ha^{-1} (Orquidea farm), from which less of 15% are natural forest. Pest and diseases are controlled efficiently under a sustainable management.

Three commercial fields on each farm with different geographical orientation (to minimize the effect of radiation) were chosen as experimental plots. The plots designated as "new" were commercial coffee production areas with 5 years of establishment, those designated as "medium" were 10 years old and the "old" plantations were those over 20 years of coffee monoculture.

Four species of aromatic plants (sage, oregano, basil and spearmint) and one control without herbs were tested as intercrops in three variably-aged coffee systems. The

treatments were planted between coffee rows. Randomized complete block design with four repetitions of treatment per plot were evaluated. Areas of 12 m^2 (6m x 2m) were de-marked as experimental units, leaving spaces of 8m^2 (4m x 2m) between treatments. Commercial seeds (Sperli) from spearmint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), sage (*Salvia officinalis* L.), and oregano (*Origanum vulgare* L.) were germinated in trays under homogeneous conditions. Four weeks old plants were planted in the field, between rows of coffee, at a density of 4 plants per m^2 (Figure 7).

To evaluate the effect of the herbs on coffee growth variables, four plants in each treatment were marked and their growth variables taken at defined intervals. Two primary plagiotropic branches (10^{th} and 20^{th}) from the upper third of the plant canopy were tagged in each treatment unit for periodical sampling of length and number of leaves. Growth parameters on coffee were evaluated every 2 months. Samples of coffee beans from experimental units were collected separately and dried to moisture content between 10 to 12 %, and used for quality control.

Six fertilizations were applied to the herbs, the first at 500 g/plant of compost at transplantation, the second three weeks after transplanting (50g/plant 46-0-8) and the remaining four after every harvest, using 100ml/plant of soluble commercial fertilizer (Hydro 17-17-17). No pest or diseases control was done on the treatments. Neither complementary agronomical management was required. The coffee plants were pruned after the first harvest (as in traditional management) and fertilized two times per year with soluble commercial product (200 ml/plant Hydro 46-15-15). No agronomical management was made and *Hypothenemus hampei* controlled by pheromones traps (17 traps/ ha^{-1}).

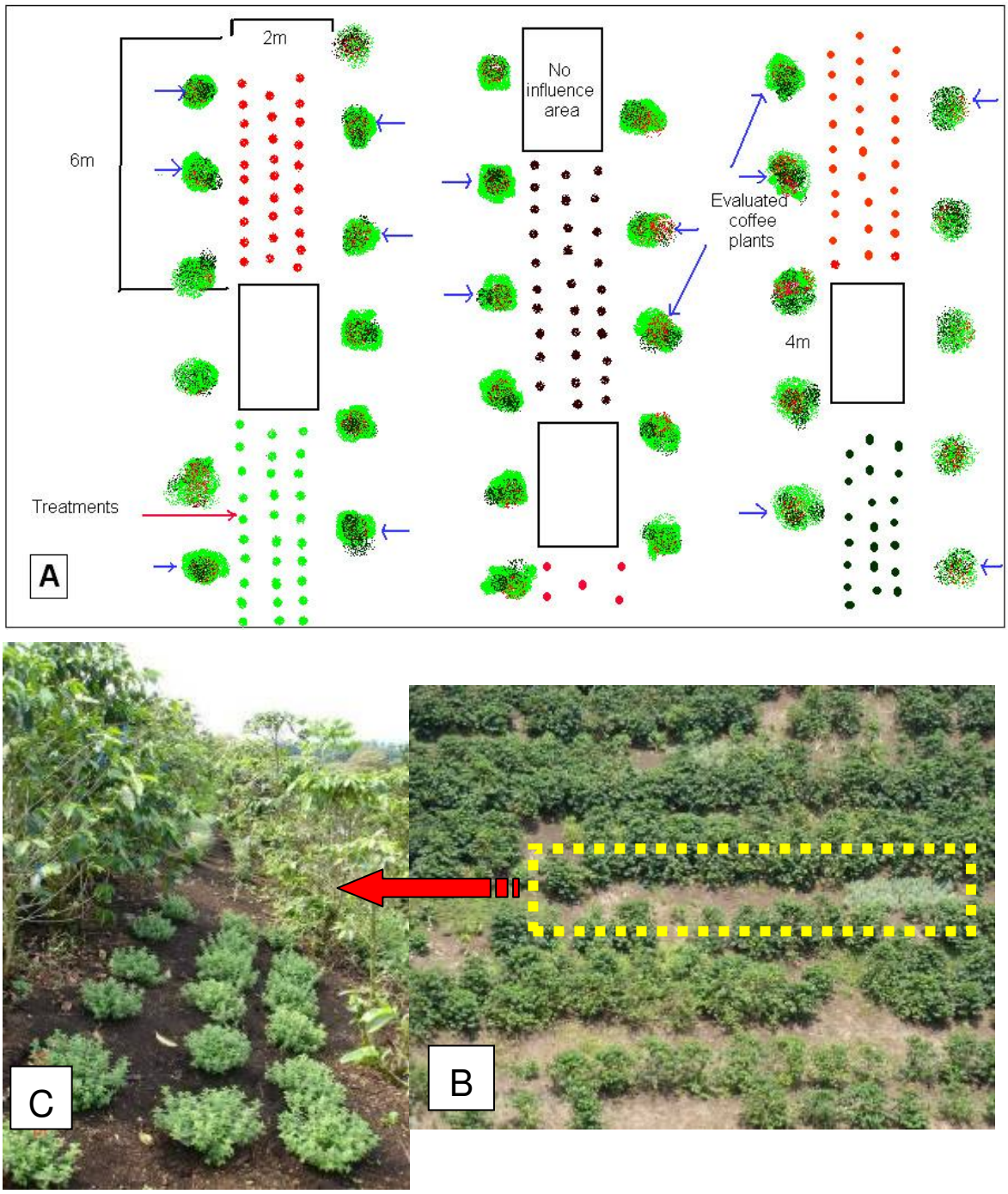


Figure 7: Experimental design and distribution of treatments in the plots in Puebla, Mexico

A: Schema of the experimental design.
B: Distribution of the blocks in the coffee production fields.
C: Distribution of the treatments in the plots.

The following factors were analyzed:

Factor A: Influence of aromatic plants on growth development of coffee. The following treatments were evaluated: A1: coffee without aromatic plants; A2: coffee with basil; A3: coffee with spearmint; A4: coffee with sage; A5: coffee with oregano. The following variables were analyzed: Plagiotropic growth (cm) and vegetative growth per marked branch (cm); appearance of new leaves per plant and quality of coffee.

Factor B: Effects of accumulation of caffeine according to age of the coffee plot on yield of aromatic plants. The treatments evaluated were: B1: New plot (5 years of establishment); B2: Medium plot (10 years of establishment); B3: Old plot (over 20 year of establishment).The yield of herbs was measured and analyzed per m² during the productive stage of the plants.

For the statistical analysis of data, SPSS 12.0 was used. The data was subjected to ANOVA and when the F test was significant, the means were compared using Tukey's test at 95 % confidence.

3.2.2 Effect of aromatic plants in coffee cup quality

For the cup quality test, an assessment of perception of coffee quality was evaluated. A sensorial evaluation (cup tasting) of the coffee form the different treatments were done by 3 professional tasters at a private coffee roaster (Coffee Star), in Berlin, Germany, in June 2006. A panel of experienced cup tasters was served coffee made from different sample plots. Samples of 5 kg were collected randomized from the treated coffee plots. All the samples were dried to 12 % humidity and then roasted and milled uniformly. The major taste and flavour attributes, aroma, body, bitterness and acidity were scored using the beverage quality denominations ranging from 0 to 7 where 0 = nil, 1 = very light, 2 = light, 3 = medium, 4 = medium-strong , 5 = strong, 6 = very strong and 7 = too strong. An additional, overall preference score for beverage quality is based on the above attributes and also ranged from 0 to 7, where 0 = unacceptable, 1 =very bad, 2 = bad, 3 = regular, 4 = acceptable 5 = good, 6 = very good and 7= excellent.

All the coffees were prepared in advance by pouring boiling water (150 mL) directly onto roasted and ground coffee (12 g; mild roast; fine ground) contained in a small cup and performing sensory (smell, flavour) evaluation after a few minutes. The data were collected and evaluated using Stat graphs 5.1.

3.3 Complementary studies

3.3.1 Post harvest management of fresh herbs

The harvests of aromatic plants were done manually in the 3rd, 5th , 7th and 9th month after crop establishment, using gardening scissors previously disinfected with a 5.5 % $NaClO_3$ (sodium chlorate).

The harvests were done early in the morning, avoiding humidity in the material due to the night-time's dew. The evaluated areas were randomized established (method of the square meter) and harvested in 4 repetitions per experimental unit. (Figure 8)

Figure 8: Method to estimate the yield production of herbs in Puebla, Mexico

The herbs were harvested according to commercial standards (leaf-quality, colour and size) starting up to 4 cm above the soil level. Cuttings between 20-25 cm lengths from herb's upper part were harvested and stored vertically in 60*40*25 cm plastic baskets with lateral aeration. The maximum weight permitted was 8 kg of fresh material per basket. The transport to the packaging plant was done the same day using cooling conditions. In the packaging plant the cuttings were placed on sucrose solution (20g ($C_6H_{12}O_6$) in 1 litre of H_2O) and refrigerated at 12 ºC during 4 hours for stimulating re-hydration.

To select the material, the basal leaves were removed and the branches standarized to 15 cm long for all the species.

The selected cuttings were vertically positioned in trays 60*80*12 cm and the basal part submerged in a bactericidal solution (1 % NaClO). Afterwards the material was stored in a refrigerated room at 4 ºC for 8 hours.

A pet crystal film of 1 mm of thickness was used for forming the trays and a permeable polypropylene film to seal them. The herbs were packed using an Italian filling and sealing machine, suitable to pack under modified and no-modified atmospheres as well as under vacuum conditions, Model SOUL 320.

In our research three different treatments were evaluated:

P1: Modified atmospheres packaging (MA). The process begins with the thermo-formation of the tray, after the plants are weighted and positioned a later time addition of 80 % carbon dioxide, 10 % nitrogen and 10 % oxygen were injected followed by the sealing.

P2: Vacuum packaging. The thermo formation of the tray is followed by weight and positioned of the herbs. A vacuum pump extracts the gases from the packing before the sealing.

P3: Control packaging. The trays were formed and filled with the herbs in the same form as described in the previous treatments. No modification of quantity and quality of gases content in the trays were made before the sealing.

All the treatments were maintained under refrigerated conditions at 4ºC during the evaluation period. The table 4 shows the relation of fresh material per specie.

The evaluations were made with an interval of 24 h, calculating the percentage of leaf damage per species. For the experiment conditions no light intensity were measured or other factors evaluated. The shelving conditions were simulated as commercial ones.

Table 4: Distribution of plant material in MA packaging according to species

Specie	Biomass weight g.	Package Weight g.	Total weight
sage	90	18.5	108.5
basil	50	18.5	68.5
spearmint	80	18.5	98,5
oregano	80	18.5	98.5

The herbs fresh material packaged in every tray was calculated and weighted according to physiological properties of each specie (size and weight of branches) and available space in the tray.

For the statistical analysis of data, the statistical package SPSS 12.0 was used. The data was subjected to ANOVA and the means were compared using Duncan's test at 95% confidence.

3.3.2 Effect of volatiles from herbs on zucchini and bean growth and yield

To evaluate the potential of different concentrations of essential oils as growth stimulants of vegetables, an experiment was done during the summer of 2006 at in the Investigation Unit of University of Bonn, located in Wesseling, Germany. Zucchini (*Cucurbita pepo* L. cv *Diamant)* and beans (*Phaseolus vulgaris* L. cv *Jutta)* were established as experimental crops, germinated from commercial seeds (Sperli seeds). Essential oils (Roth) from oregano, spearmint, basil, and sage were diluted with tap water to obtain concentrations of 0.1 %; 0.3 %, and 0.5 %. To homogenize the mixture, 2 ml of a commercial detergent (Ivraxo soft) at 1 % was used. The different concentrations as well as a control (Tap water) were sprayed on evaluated species.

Two experimental units, each 500 m^2, were divided in a randomized design with four blocks per species. Four week-old plants that had germinated under greenhouse conditions were transplanted to the field. The seeding density was 4 plants per m^2. The treatments were randomly distributed with four repetitions per block. Spaces among treatments of 8 m^2 were left. Weekly applications using 40 ml of VEOs dilutions per plant were made over 2 months. The applications were done in the morning, avoiding high temperatures and

windy conditions, using commercial 1 litre sprinklers. Drop irrigation systems were established and weeds were controlled on ridges using black polyethylene mulch.

Plants were fertilized twice within a 30-day interval during the evaluation. A soluble fertilizer (Hydro 17-17-17 (50 kg/400 l)) was applied through the drip-irrigation system.

The evaluated essentials oils were oregano, basil, mint and sage applied in different concentrations (0.1 %, 0.3 % and 0.5 %).

Number of fruits per plant and their weight as well as fruit diameter and length were measured, using a sampling of 4 repetitions per treatment on marked plants.

The harvest was done weekly and according to commercial standards of size and maturity of fruits.

For the statistical analysis of data, the statistical package SPSS 12.0 was used. The data was subjected to ANOVA and the means were compared using Duncan's test at 95% confidence.

4 RESULTS

4.1 Accumulation and release of caffeine by aromatic plants

The experiments on caffeine uptake revealed that all the species were able to absorb caffeine and accumulated it mainly in the roots. No evidence of detoxification products or of a possible biodegradation of caffeine after uptake was detected by chromatography.

All herbs absorbed similar amounts of caffeine over the incubation period of 24 hours. The highest uptake capacity was shown by sage, with 6.92 µmol caffeine absorbed in 24 hours (Table 5).

Table 5: Dynamics of caffeine uptake and release by aromatic plants

Treatment	Sample taken from:	Caffeine Uptake µmol	Caffeine Release µmol	Caffeine Balance µmol
basil 24 h	root	5.30± 0.4	4.91± 0.3	0.39± 0.1
	leaves and shoot	0.00		
	medium	133.30 ± 0.3		
	total	138.60± 0.3		
basil 48 h	root	4.99± 0.3	5.72± 0.6	-0.73± 0.2
	leaves and shoot	0.00		
	medium	168.30 ± 0.3		
	total	173.35 ± 0.3		
sage 24 h	root	6.92± 0.4	0.33± 0.1	6.59± 0.4
	leaves and shoot	0.00		
	medium	149.10 ± 0.3		
	total	156.20 ± 0.3		
sage 48 h	root	9.62± 0.3	0.14 + 0.62*± 0.3	8.86± 0.4
	leaves and shoot	0.00		
	medium	132.10 ± 0.3		
	total	141.70 ± 0.3		
spearmint 24 h	root	4.73± 0.4	4.27± 0.6	0.46± 0.2
	leaves and shoot	0.00		
	medium	171.65 ± 0.3		
	total	176.30 ± 0.3		

spearmint 48 h	root	5.53± 0.3	0.70 +0.39*± 0.3	4.44± 0.3
	leaves and shoot	0.00		
	medium	145.91 ± 0.3		
	total	151.40 ± 0.3		
oregano 24 h	root	5.00± 0.8	1.65± 0.2	3.35± 0.4
	leaves and shoot	0.00		
	medium	145.35 ± 0.3		
	total	150.30 ± 0.3		
oregano 48 h	root	9.45± 0.3	0.21 +0.41*± 0.2	16.14± 0.3
	leaves and shoot	7.31± 0.5		
	medium	125.90 ± 0.3		
	total	142.75 ± 0.3		
*additional amount of caffeine found in herb's biomass after 72 h of release				

Over the next 24 h, sage and oregano continued to accumulate caffeine whereas in spearmint, the concentration of the compound increased only slightly. No further absorption was observed with basil. Caffeine was only detected in the roots of the aromatic plants, except in oregano, where 7.31 µmol were found in the shoots after 48 h of exposure.

To test the possibility that absorbed caffeine is released back into the environment once the roots are not longer in contact with this allelochemical; the plants were transferred to tap water for 24, 48 and 72 h. The analysis of the compounds found in the tap water after 72 hrs indicated a complete release of caffeine absorbed by basil (both treatments: 24 and 48 h incubation with caffeine). For spearmint, a complete release was observed only following the 24 h-caffeine treatment, whereas after the 48h-treatment, less than 20 % of the absorbed caffeine was detected in the tap water fraction. Oregano released about 33% of the caffeine it had absorbed over the 24 h period, but only 2-3% when it had 48 h to absorb it. The lowest amount was released by sage (about 5% after the 24 h-treatment and 1.5% after the 48 h-treatment). Six percent of the absorbed caffeine appeared in the shoots of the sage incubated with caffeine for 48 h, after transferring the plants to tap water. After 72 h in tap water, 7% and 5% respectively, of the caffeine absorbed by spearmint and oregano over 48 hours was found in the shoots.

The overall balance of caffeine uptake and release indicates a complete release of all absorbed caffeine by spearmint after the 24 h treatment with caffeine and by basil after both caffeine-treatment periods (24 and 48 h). With spearmint (48 h-treatment), sage and oregano (both treatments) a large portion of the caffeine absorbed was neither released into the tap water nor was it extractable from roots, stems or leaves.

4.2 Effects of volatiles on stomata aperture of coffee leaves

Stomata of the control coffee plants were closed or showed minimal opening after being placed in the exsiccator for 24 (more than 50%: 0 - 0.67 µm), 48 (75 %: 0 - 0.67 µm) and 72 h (more than 85%: 0 - 0.67 µm). The number of closed stomata increased over time, probably a reaction to air circulation caused by ventilation or as a regulatory reaction to reduce water loss. The presence of oregano increased average stomatal apertures to 46% between 4.05 - 4.75 µm after 48 h. Pore sizes decreased after 72 h, but they were still larger than after 24 h of exposure, where more of the 50% were almost closed (0 - 0.67 µm) (Table 6).

Table 6: Stomata aperture of *C. arabica* leaves after different times of exposure to herbs volatiles. Observations were made using a 60x ocular for calibration. Stomata aperture of *C. arabica* leaves (n=50) were noted and the percentage of aperture calculated.

Data in µm	Percentage of aperture after 24h					Percentage of aperture after 48h					Percentage of aperture after 72h				
	mint	oregano	sage	basil	control	mint	oregano	sage	basil	control	mint	oregano	sage	basil	control
0,00		20			22					35	6				60
0,67		34	12		32				12	40					27
1,35		32	32	10	18				16	12		8			8
2,02		14	24	14	16				28	15		12		10	5
2,70	30		16	24	12	10	8	44	32			8		14	
3,37	34		12	30		34	14	32	12			24	10	23	
4,05	12		4	12		46	20	14				16	18	33	
4,75	16			10		6	26	8				32	42	12	
5,40	4					4	14	2			12		16	8	
6,07	4						18				36		10		
6,75											30		4		
7,42											16				

Stomatal opening was also promoted by sage volatiles (24 h 50% = 1.35 – 2.02 µm, 48 h more than 75% between 2.70 -3.35 µm and 72 h over 80% = 4.05 µm). *Mentha piperita* volatiles were most effective in promoting stomatal opening. After 24 h exposure to spearmint volatiles more than 60% of observed stomata pore size was higher than 2.70 µm, after 48 h 80% between 3.37 - 4.05 µm, and after 72 h of exposure, maximal sizes of apertures were reached (up to 6.75 µm). The long term exposure also resulted in a portion of closed (6%), obviously damaged stomata. Volatiles from basil also induced stomata opening, but prolonged exposure times did not further increase the pore sized (mean averages 24 h = 3.37 µm, 48 h = 4.05 µm and 72 h = 4.05 µm) (Figure 9).

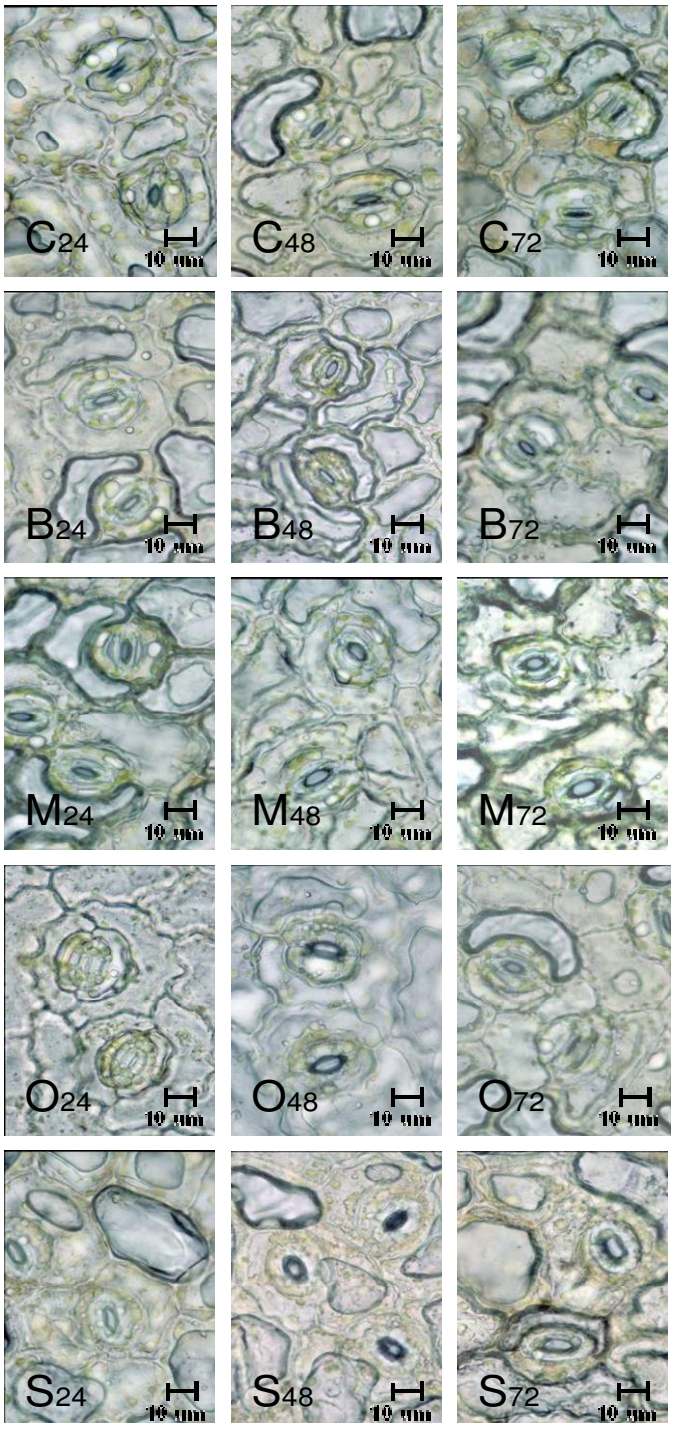


Figure 9: Photos of coffee (*Coffea arabica* L.) leaf stomata after influence of volatiles from herbs (B = basil, M = spearmint, O = oregano, S = sage) and the control (C = no treatment) by different times of exposure (24, 48 and 72 h). Size bars are 10 µm for all photos.

Mean stomatal aperture was highest and increased over the entire experimental period with spearmint. Sage volatiles had more of an effect between 48 and 72 h, than after only 24 hours. An increase of aperture (24 to 48 h) and later closured of stomata (48 to 72 h) was observed in the presence of oregano volatiles. No significant effect on stomata opening due to volatiles from basil was observed, though this meant that they stomata were open wider after 72 h than those of the control leaves (Figure 10).

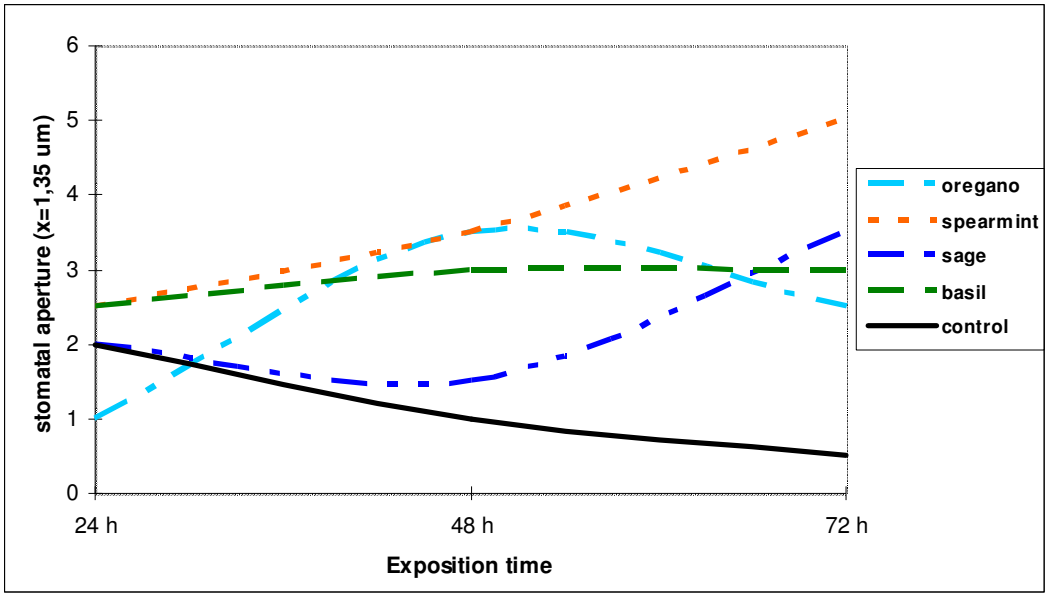


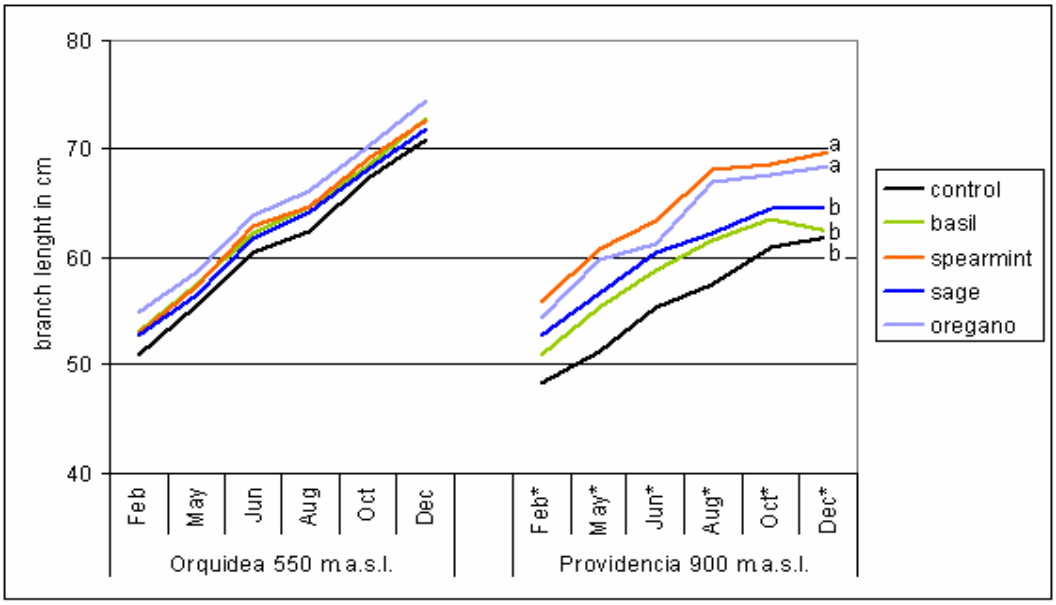
Figure 10: Dynamic of stomata opening means of coffee leaves under influence of volatiles from herbs for the evaluation period

The analyzed data shows that volatiles of spearmint, sage, oregano and basil opened the stomata of coffee plant leaves. Aperture from stomata indicates an increase in transpiration rate of exposed plants. Higher stomata aperture enhances the influx of CO_2 which is required to maintain a high photosynthetic activity.

4.3 Stimulatory effect of aromatic plants in coffee plantation

The evaluation done in Puebla, México, revealed an increase in branch length when aromatics plants were intercropped. A higher growth rate with mint, oregano (about 2

cm/month) was found when compared to control plants (1.3 cm/month). Statistical analyses provide significant differences between control and treatments where spearmint and oregano were intercropped for the evaluation periods (Feb.-Dec.) at the high altitude conditions (Providencia Farm). Thus no significant difference was observed for the other two treatments (basil and sage intercropped) the mean averages of these treatments were superior in comparison with the control during the whole year (Figure 11).



* Significance difference, $P = 0.05$

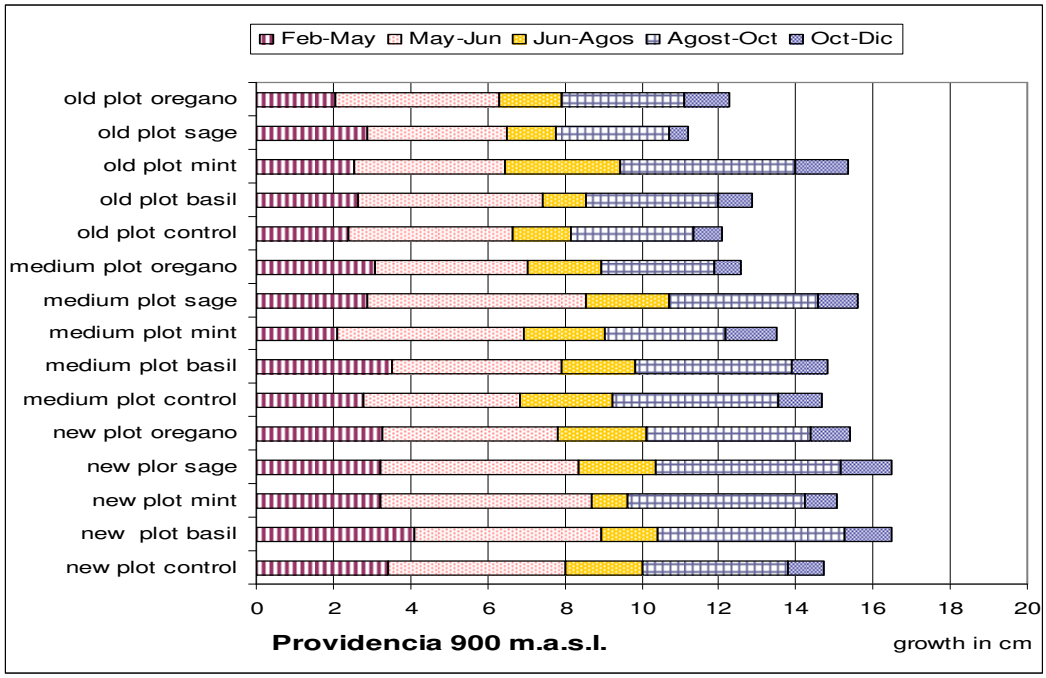
Figure 11: Branch length development of coffee (*Coffea arabica* L.) under influence of intercropped herbs

No significance differences were observed at lower altitude conditions (Orquidea Farm). However, higher means of branch growth in coffee when oregano and spearmint were intercropped suggest an important role of those treatments in plagiotropich growth of coffee branches (Figure 11). Observations in both ecological conditions (550 and 900 m.a.s.l) shows a higher rate of increase in branch length of coffee plants localized side of the evaluated plants, coinciding with the influenced area of volatiles from herbs (Figure 12).

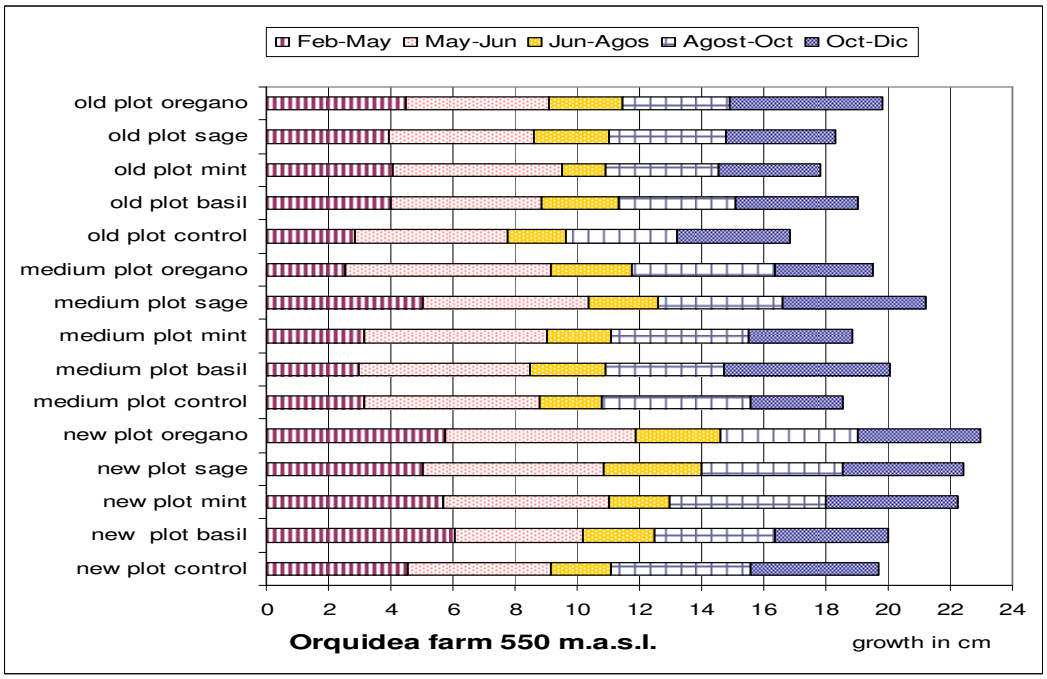
Figure 12: Localized effect of volatiles form herbs on branch growth of coffee (*Coffea arabica* L.) in Puebla, Mexico

Red circle enclosed the area of no stimulation of volatiles and with less physiological development of *C. arabica* plants. The yellow circle highlights the area of major development under direct influence of volatiles from sage (*Salvia officinalis* L.).

According to the evaluation periods, stimulated branch growth was observed in new plot (5 years of establishment), medium aged (10 years of establishment) and old plots (over 20 years of establishment). In old plots, the greatest stimulation was found with mint. Sage had a slightly negative effect. In medium aged plots, sage was most stimulatory, whereas mint and oregano were inhibitory. In new plots, oregano, sage and basil increased branch length, while mint did not have an effect on branch growth. Although some of the aromatic species reduced branch length slightly, the positive effects prevailed over the vegetation period from February to December.



A



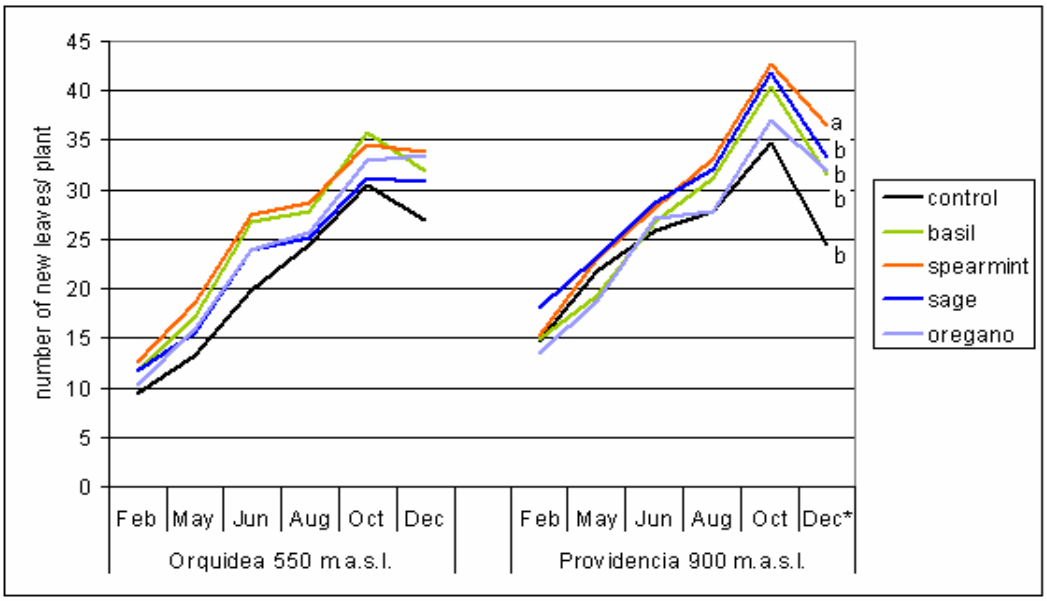
B

Figure 13: Developing of coffee branches according to evaluation periods and ecological conditions A: High altitude 900 m.a.s.l. and B: Low altitude 550 m.a.s.l

New plot: 5 years of coffee production
Medium plot: 10 years of coffee production
Old plot: over 20 years of coffee production

The better growth rate observed in the different experimental units during the period between May-June and August-October, coincide with the highest average of rainfall periods.

A significant difference for the average of number of new leaves was found in December 2005 at high altitude (900 m.a.s.l. in Providencia Farm) for the treatments with spearmint (36,50 leaves/plant)in comparison with only 24,31 leaves per plant of the control. However, no differences were found for the other species (basil oregano and sage) at these evaluated conditions the average of new leaf appearance in coffee per plant was higher in treatments with aromatic herbs intercalated than in the control. For low altitude, means appearance of newly members of coffee leaves was higher in comparison with the control during the evaluation period, thus no significant difference found (Figure 14).



* Significance difference, $P = 0.05$

Figure 14: Appearance of new leaves per plant in coffee (*Coffea arabica* L.) under influence of intercropped herbs

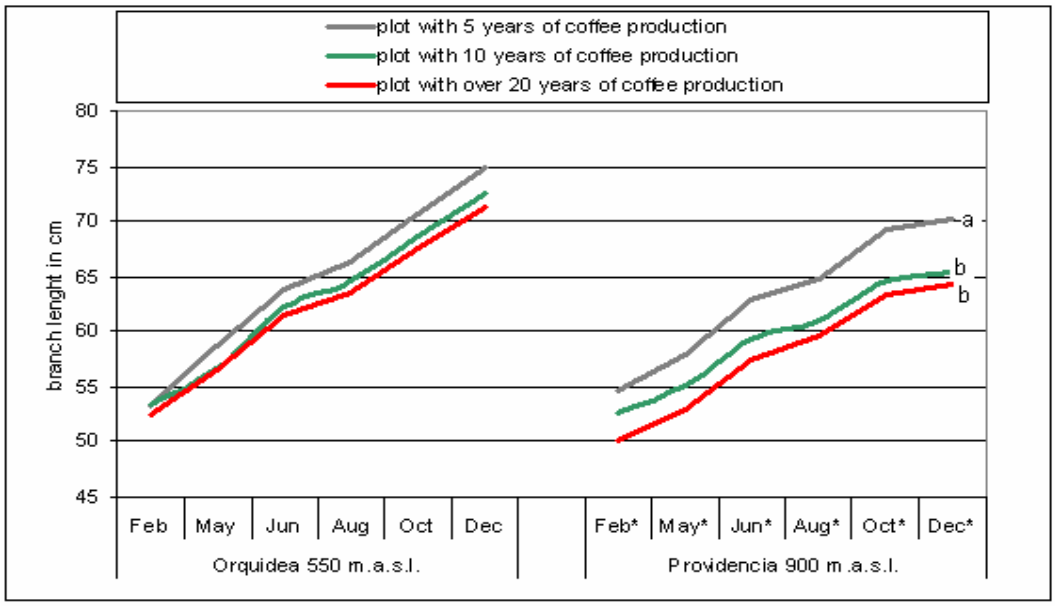
A decrease in the development of new leaves was observed in October, coinciding with the start of fructification and constrain of nutrient availability for the vegetative growth.

Highly significant growth stimulation (plagiotropic growth and appearance of new leaves) was observed when coffee was intercropped with aromatic plants for all experimental plots

when compared to the corresponding controls, though these differences differed from one system to another.

4.4 Effect of plot age on coffee growth

A decrease in plagiotropic growth in relation with the age of the plot was observed. The average branch growth was less in production systems that had been under monoculture for over 10 years than in those that had been establishment within the last 5 years (Figure 15).

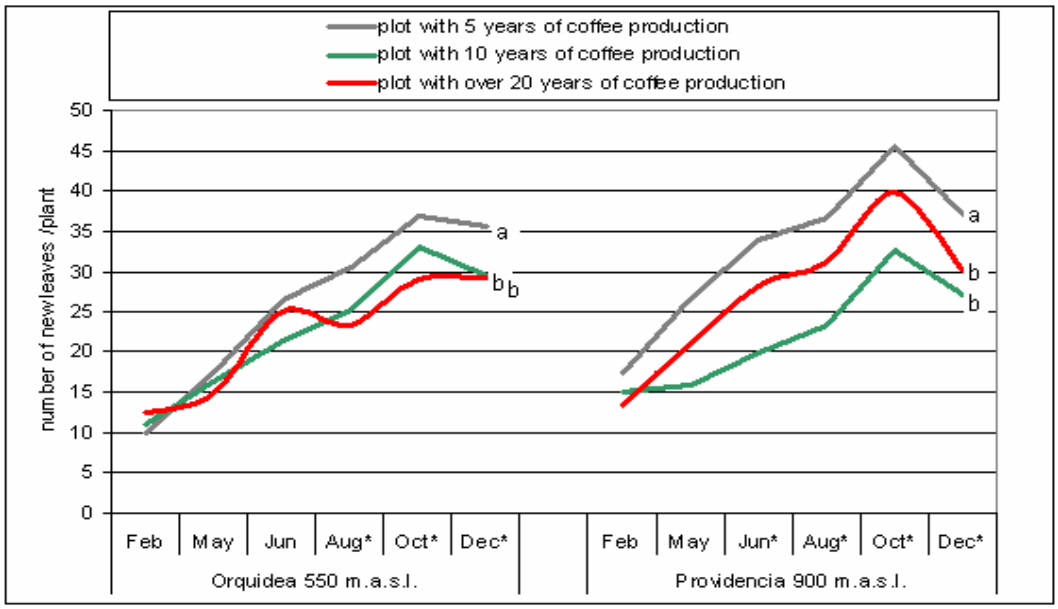


* Significance difference, $P = 0.05$

Figure 15: Branch length development in coffee (*Coffea arabica* L.) according to age of the production system

Significantly differences were observed (Feb.-Dec.) for the means of branch length of plots with 5 years of production under high altitude conditions when compared to those of older plots (10 and over 20 years of production).

Development of new leaves was also related to the age of the plots. The decrease in the development of new leaves in October, as previously noted, was found in plots of all ages (Figure 16).



* Significance difference, $P = 0.05$

Figure 16: Appearance of new leaves per plant in coffee (*Coffea arabica* L.) according to age of the production system

Significant differences were observed for the number of new leaves developed, for the last six moths of the year, under both ecological conditions (550 and 900 m.a.s.l). The best average leaf production was seen in the plots with 5 years of production.

The results on this study suggest that plot age is a limiting factor in coffee growth. The best growth rate seen in the different experimental plots, during the period between May and June coincide with the highest average of rainfall periods and growth rate decrease coincides with the beginning of the fructification period (October). In summary, branch length and the number of new coffee leaves formed were negatively influenced by age of plot and the possible accumulation of caffeine in older coffee soils.

4.5 Effect of aromatic plants in coffee cup coffee quality

Coffee quality is understood as a combination of physical characteristics like bean size and weight, absence of physical damage and chemical characteristics such as caffeine, trigonelline, sucrose and chlorogenic acid content, as well as cup quality, which is a combination of aroma, body, acidity, bitterness and others parameters. Among these

criteria, flavour is the most important criterion for coffee quality evaluation, and the one most widely employed in coffee trading (Franca *et al*., 2005; Farah *et al*., unpublished data). Figure 17 presents the impressions that professional coffee testers from Coffee Star Berlin had of the coffee produced under intercropped systems, using the various herbs tested.

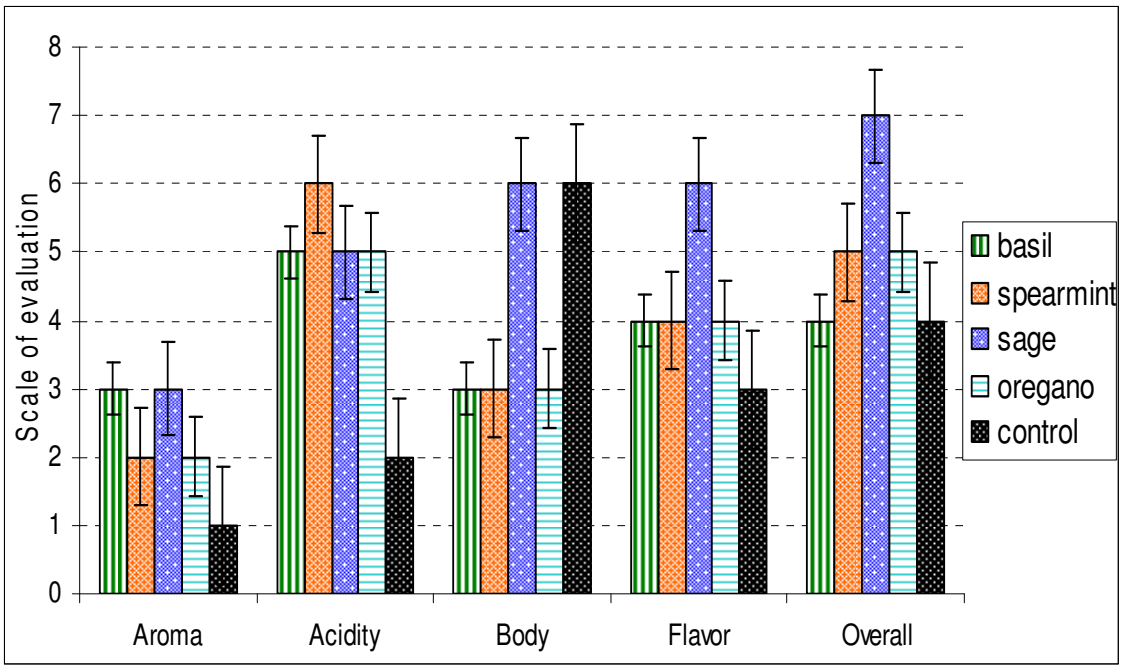


Figure 17: Effect of interaction of herbs in Coffee (*C. arabica* L.) cup quality. Impression of professional tasters, Coffee Star, Berlin (2006)

The best cup quality was obtained with coffee from samples intercropped with sage. The overall impression was generally better when coffee was obtained from samples intercropped with aromatic plants than from control plots. As opposed to other features looked at, body was below or equal to that of the control in samples harvested from coffee plants intercropped with herbs. The presence of spearmint also resulted in a higher acidity compared to the other treatments and the control. All aromatic plants had a positive effect on coffee aroma and flavour. The cup quality of coffee could be improved when coffee plants are intercropped with oregano, spearmint, basil and sage.

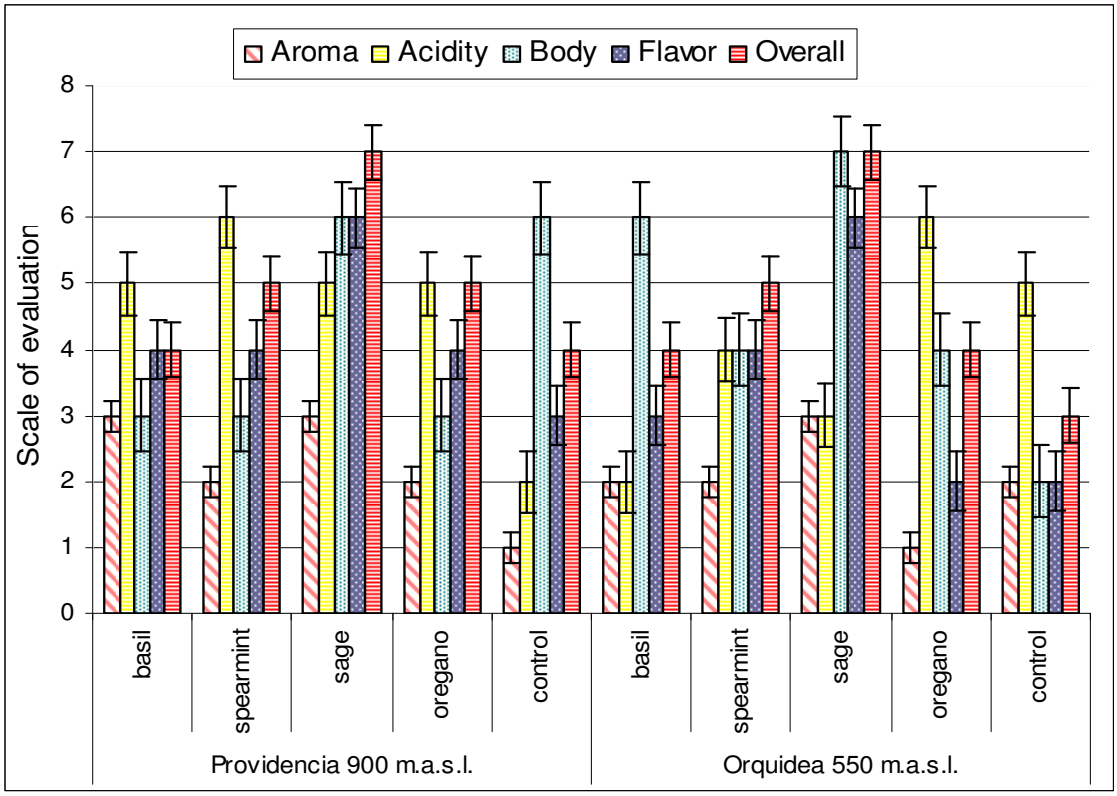


Figure 18: Effect of volatiles from herbs in coffee (*Coffea arabica* L.) cup quality under different ecological conditions.

According to the ecological conditions evaluated (low and high altitude) the overall impression was better when coffee was obtained from samples intercropped with aromatic plants; the best cup quality was reached with sage intercropped in both altitudes when compared to the control. However, in samples harvested from coffee plants intercropped with oregano at low altitude, aroma was below that of the control. Under the same ecological conditions, the presence of oregano also resulted in higher acidity, desirable in coffee with low acidity such as Mexican coffee. In all other aromatic plants and both controls, acidity was relatively low. All aromatic plants had a positive effect on coffee body, though the control under high altitude conditions was deemed best, closely followed by sage. The results show that exposure to aromatic plants is obviously a factor that influences the composition of aroma precursors in coffee by still unknown mechanisms. As expected, all cup quality characteristics evaluated were better under high altitude conditions than under low ones. The sensorial evaluation of coffee, called its organoleptic

quality or, more simply, its "cup quality", depends on environmental and genetic factors, as well as on agronomic production of the crop and its post-harvest management.

4.6 Results on yield and post harvest management of herbs

Experiment results and field observations show negative influence of caffeine accumulation in soil on the production yield of basil, oregano, spearmint and sage. Sage, oregano and basil revealed to be the less affected species in the three production systems evaluated and high altitude conditions. Under plots of 10 year of coffee establishment, basil and sage show better average of yield production in comparison with the other two crops, thus demonstrating that they can adapt well to coffee plantations by the means of a tolerance mechanism to the potentiality toxic effects of caffeine (Figure 19).

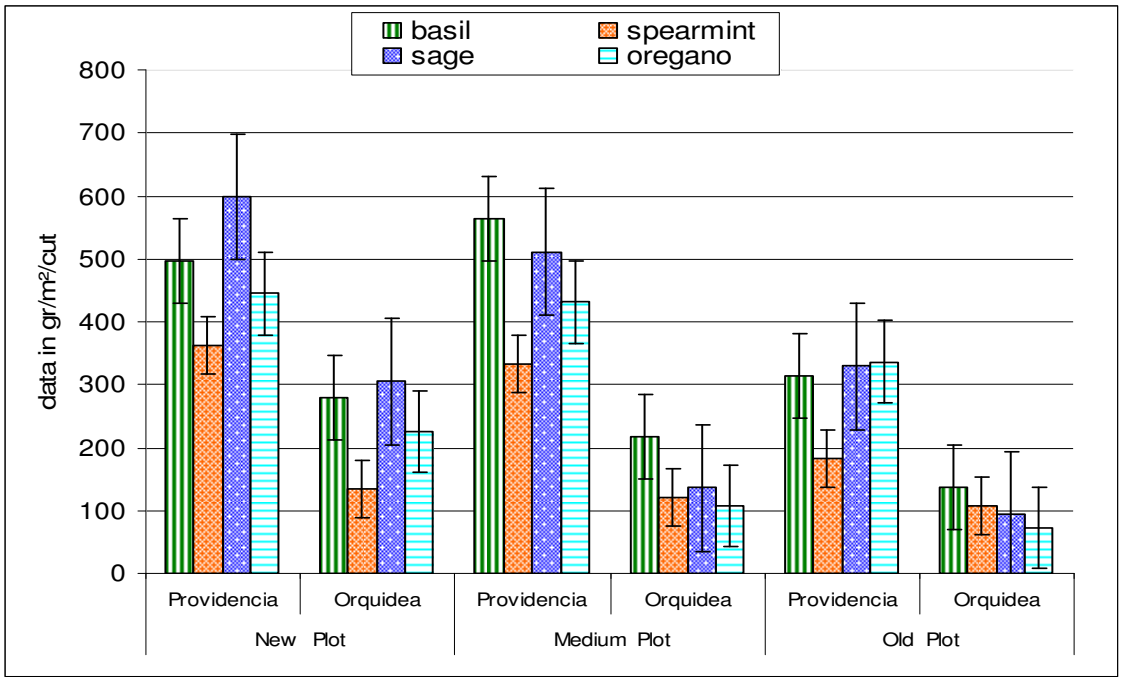


Figure 19: Yield production of herbs under different age coffee systems in Puebla, Mexico

The negative interaction in soils with more than ten years of coffee monoculture has been previously reported by Weinberg and Bealer (2001), due to accumulation of soluble form of caffeine in the soil. Under old coffee system the yield production of all species decrease

and demonstrate that thus a involved mechanism to tolerate certain levels of caffeine, higher amounts of the alkaloid in the soil can be toxic, limiting the tested crops development. A notorious general result for all species is that they grow better on younger fields and at higher altitudes.

Data demonstrates that caffeine acts as a negative allelochemical to aromatic plants. Although there is a high potential of intercropping basil, sage, spearmint and oregano between coffees rows in order to obtain extra income for the idle area, evidence on this study indicates that age of the plots and accumulation of caffeine in the soil are limiting factors in the yield of aromatic plants.

4.6.1 Results of modified atmospheres as packing solution for herbs

Results showed all herbs extend their storage life better under modified atmospheres or vacuum conditions than traditional management (control). Storage life of basil, oregano, spearmint and sage were prolonged up to 12 days under modified atmospheres being the best treatment with losses below 20% (level of damage permitted in fresh herbs) in comparison with more than 30% for vacuum conditions and up to 60% losses in the control. The rate of product deterioration expressed as leaf yellowing and blackening, increased substantially for this treatment just after 18 days of evaluation (Figure 20).

Figure 20: Photo of sage, oregano and spearmint under modified atmospheres = MA packaging after one week (superior part of the picture) in comparison with the control = C (inferior part) for the same evaluation period

In the vacuum storage trial basil and spearmint responded favourably with damage average below 20% during the first two weeks of evaluation. The vacuum packaging appeared to have little effect on the storage life of oregano and sage, as it performed just well the first week of evaluation, increasing leaves damage up to 50% for later periods (Figures 21; 22; 23; 24).

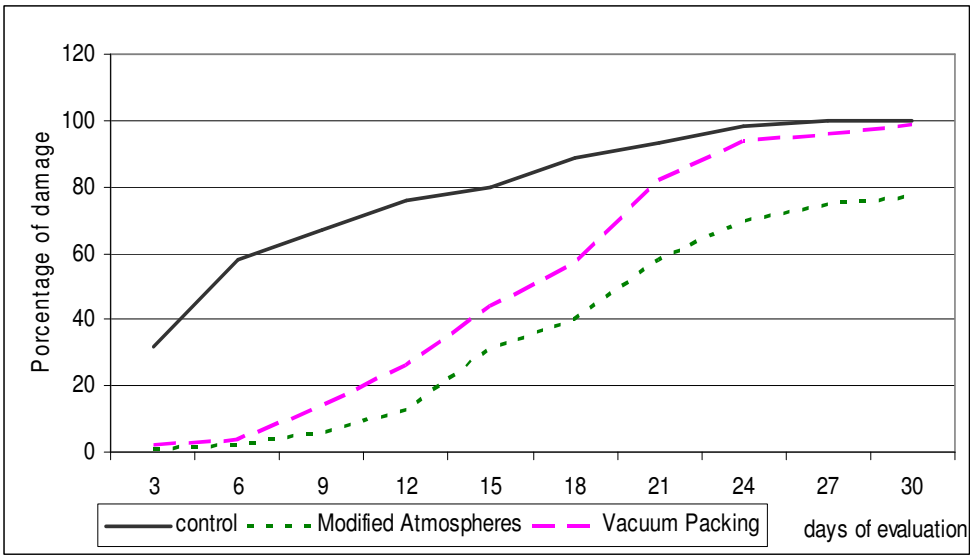


Figure 21: Effect of packing method in Shelf Life in basil (*Ocimum basilicum* L.)

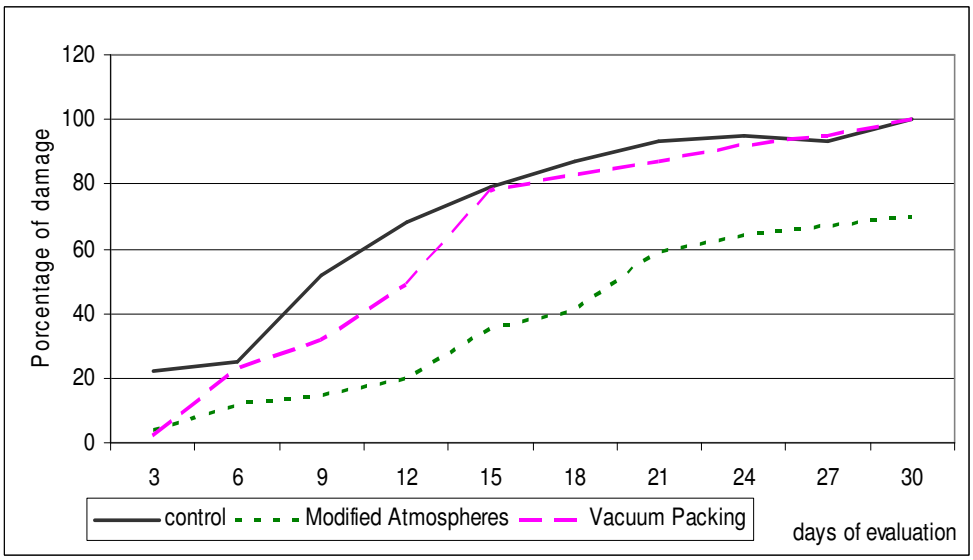


Figure 22: Effect of packing method in Shelf Life in oregano (*Origanum vulgare* L.)

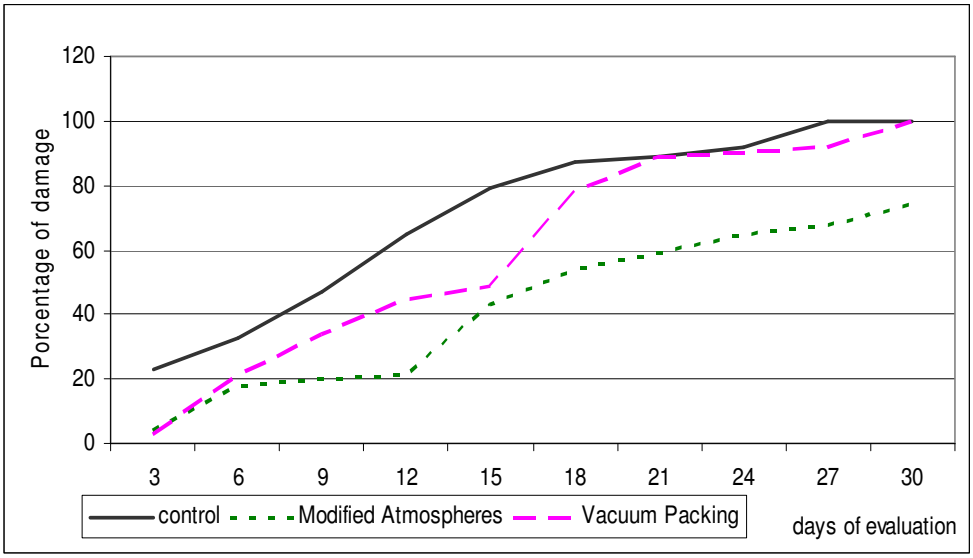


Figure 23: Effect of packing method in Shelf Life in sage (*Salvia officinalis* L.)

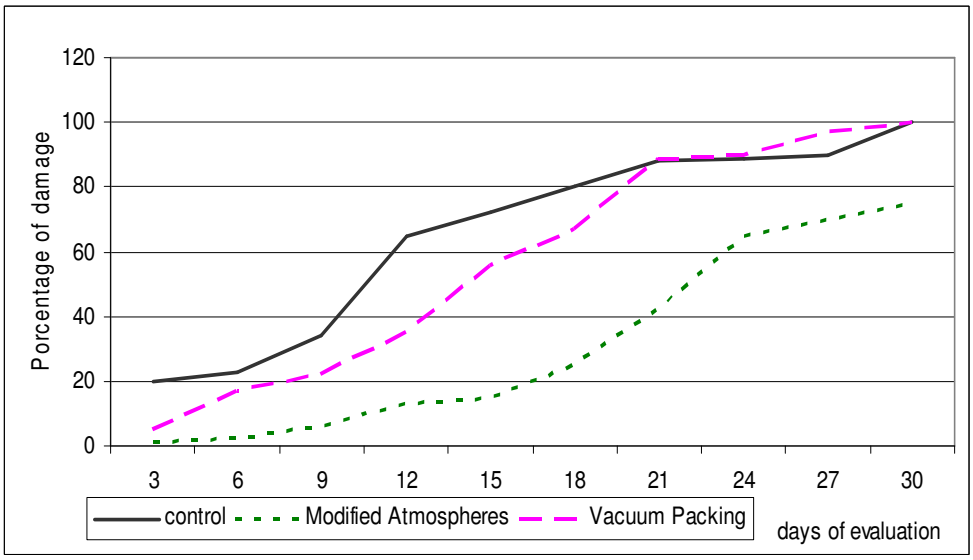


Figure 24: Effect of packing method in Shelf Life in spearmint (*Mentha piperita* L.)

For the control more than 20% of leaf yellowing and blackening of leaves and steams for all the species just after the third day of shelf life, show the limiting of this treatment as commercializing solution for fresh herbs.

4.7 Stimulation of yield and growth in zucchini and beans by volatiles from oregano, sage, spearmint and basil

4.7.1 Results for zucchini (*Cucurbita pepo* L.)

Zucchini growth variables analyzed revealed no significant differences between the different essential oils. In general, average yields were greater in the presence of essential oils under all concentrations tested (Figure 25). The relation between diameter and length of the fruits were concurrent and no physiological changes according to treatments were observed.

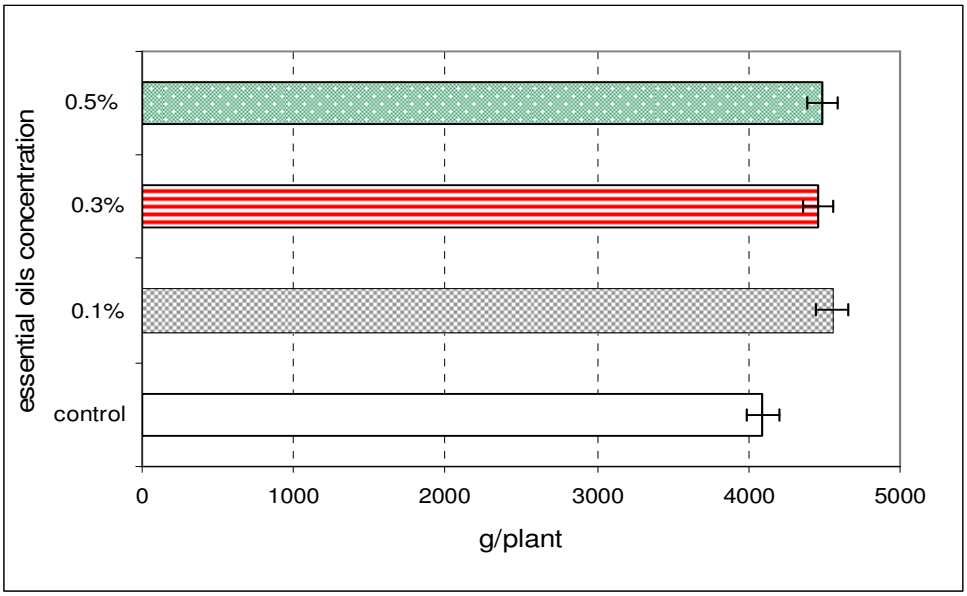
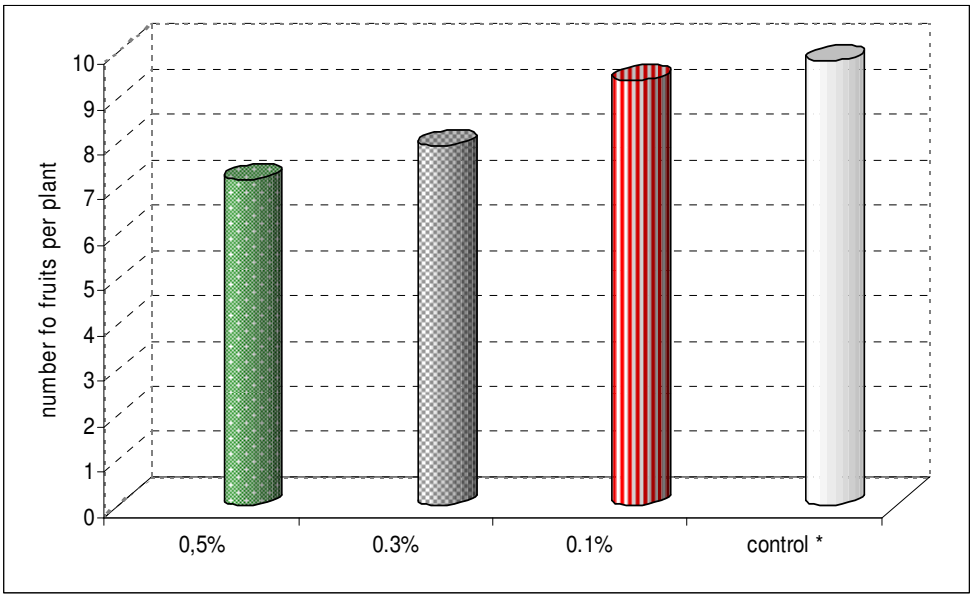


Figure 25: Effect of concentrations of volatile essential oils on yield production of zucchini (*Cucurbita pepo* L.)

The essential oils significantly increase the yield of zucchini. Average zucchini yield reached 4550 g/plant in the treatment with 0.1 % of essential.

The other two treatments (0.3 and 0.5 %) showed a slightly increase in yield when compared to the control (about 10%). Increased growth in all the treatments compared to control provides evidence of the presence of a certain plant growth-stimulating factor.

A decrease in the number of fruits per plant was shown with high concentrations of oils. Significant differences for this variable were observed, with a higher average number of fruits for the control in comparison with that of the treatments (Figure 26).



* Significance difference, *P*=0.05

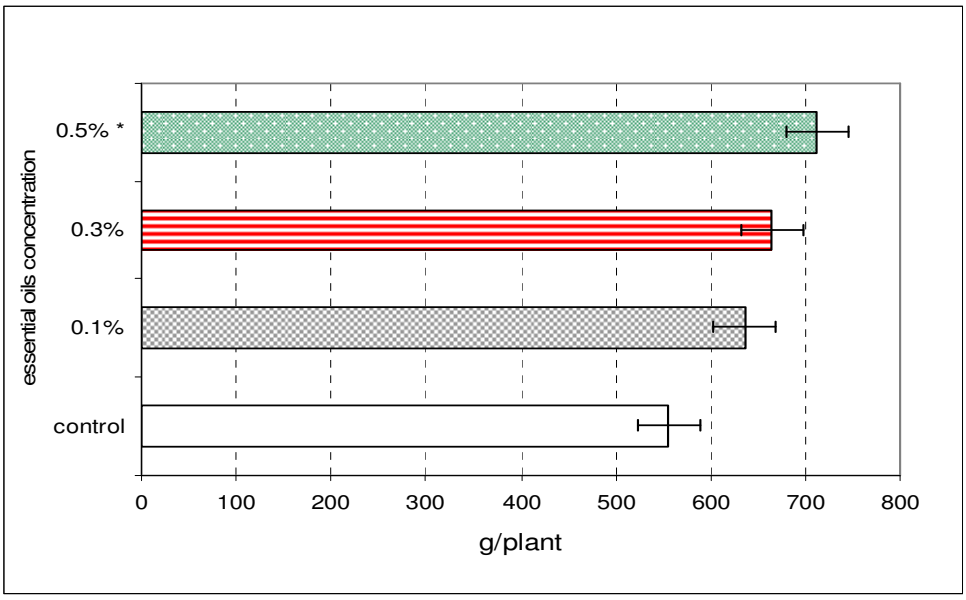
Figure 26: Effect of concentrations of volatile essential oils on the number of fruits per plant in zucchini (*Cucurbita pepo* L.)

The higher average number of fruits in the control could be the result of a repellent effect from the applied treatments on pollinator insects with a notable decrease in fructification in the treated plants with increasing concentrations.

However, there was no change in form and size of fruits compared to those of the control. Neither observation on physiological changes and organoleptic properties due to applications of essential oils were found for this species.

4.7.2 Results for beans (*Phaseolus vulgaris* L.)

Volatile essential oils also significantly stimulated yield production in beans. Significance differences were found when 0.5 % essential oils were applied (Figure 27).



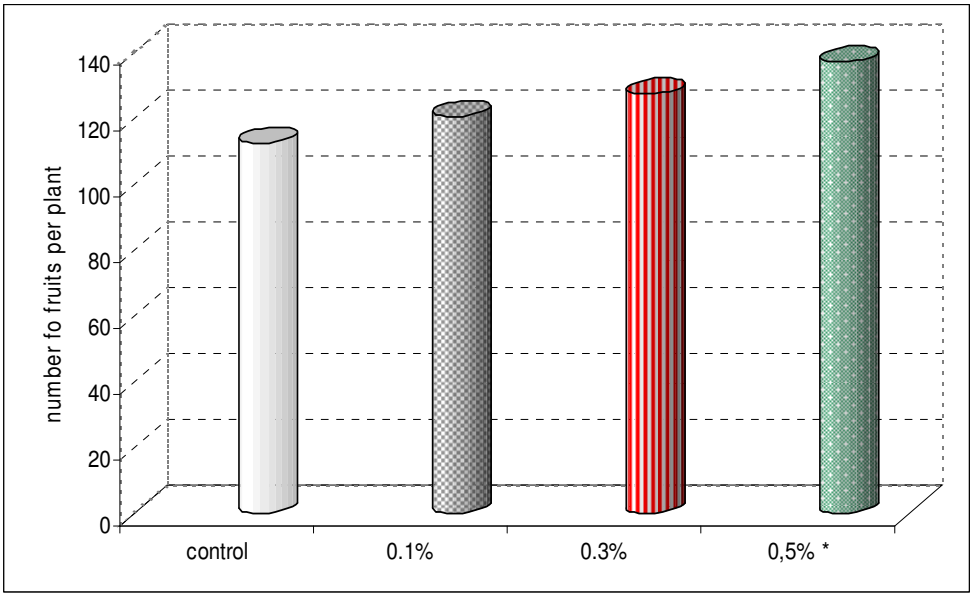
* Significance difference, $P = 0.05$

Figure 27: Effect of concentrations of volatile essential oils on yield production of beans (*Phaseolus vulgaris* L.)

The essential oils increased the yield of beans in all the evaluated treatments. A significantly high bean yield of 721 g/plant was obtained in the treatment with 0.5 % of essential oils. The control plants only yielded 555 g/plant. However, growth stimulation in beans for the other two treatments evaluated was not statistically significant, with yields only 15-20 % higher than those of the control.

Essential oils increased the number of pods produced by bean plants, significantly so when 0.5 % essential oils were applied.

The increase was proportional to the dosage sprayed being better the average in the treatments with high concentrations of oils (Figure 28).



* Significance difference, $P = 0.05$

Figure 28: Effect of different concentrations of volatile essential oils on number of fruits per plant in beans (*Phaseolus vulgaris* L.)

The data obtained for the number of fruits in beans, shows that bean flowers are capable of self-pollination although cross-pollination can and does occur to varying degrees, depending upon the cultivar and the pollinator population. However, the repellent effect of the essential oils seen in zucchini was not a limiting factor for production in beans.

5 DISCUSSION

5.1 Potential uptake of caffeine by herbs

Plot age and accumulation of caffeine in the soil are limiting factors for coffee growth. Antimicrobial activity of caffeine may reduce catabolism of the alkaloid in the soil, prolonging its retention and increasing accumulation in the surrounding areas. Since most of the coffee roots are developed in the upper soil layer, immediately under the tree's own litter, autotoxicity can be manifested (Friedman and Waller, 1983a).

This fact may provide an explanation for the worldwide phenomenon of early degeneration of coffee plantations and could be responsible for shortening the productive lives of coffee plants and decreasing the production of new leaves in coffee plants. Caffeine uptake by coffee seedlings and translocation of the alkaloid to all parts of the seedling was described by Waller et al. (1986). As reported by Mazzafera and Gonçalves (1999), purine-related alkaloids, such as caffeine, are translocated in the plant and found as constituents of xylem sap. Absorption and translocation was regarded as a mechanism to remove caffeine from the root tips, which are more susceptible to the allelochemical than shoots. Absorption of caffeine by other plants has not been investigated. The bioassays revealed the caffeine uptake capacity of aromatic herbs. However, it is likely that basil, sage, mint and oregano have a different method of taking-up and translocating the compound to the shoot than coffee does.

The capacity of aromatic species to detoxify exogenously supplied caffeine was analyzed by quantification of the uptake and release of the alkaloid. The total release of caffeine by basil after 24 and 48 h of incubation indicates that active uptake is realized late in comparison to spearmint, and especially to sage and oregano. The data suggest a potential caffeine metabolization through *S. officinalis* and *O. vulgare* since a difference greater than 80% in the uptake-release balance of the alkaloid was observed after 24 and 48h, and only a small amount of the allelochemical was extracted from the biomass after 72 h of release. The metabolism of purine nucleotides and purine alkaloids (e.g. caffeine) has been reviewed in tea and coffee plants by Suzuki *et al*. (1992). Xanthosine is the first methyl acceptor from S-adenosylmethionine in caffeine biosynthesis, and is also metabolized by a purine degradation pathway via xanthine. In *Coffea* plants, the rate of caffeine synthesis and turnover (i.e. biodegradation and/or biotransformation to xanthine or to methyluric

acids) differs markedly among species and remains uncertain. Little is known about the caffeine biodegradation pathway and further research that can complement results from this study remains to be done.

The aromatic plants and probably many other species which have still to be investigated, may be able to diminish soluble caffeine in the soil of coffee plantations. As intercrops able to actively take-up caffeine, sage and oregano may help in retarding the early degeneration of coffee plantations.

Results from the bioassays are similar to field results, where herbs grew better under low caffeine conditions (in fields with 5 years of coffee production) revealing potential limiting factors on herb yields in old plantations (over 20 years of coffee monoculture) due to a higher accumulation of caffeine in these areas and greater potential uptake by herbs of the alkaloid. The possible ability of the aromatic plants to metabolize the compound increases their value as intercrops.

5.2 Effect of volatiles on coffee leaves stoma aperture and coffee growth

In a study by Schulz *et al*. (unpublished data), an increase of stomatal aperture by volatiles of *Lavandula latifolia*, *Artemisia camphorata* and *Mentha piperita* was observed in *Arabidopsis thaliana* leaves. Effects on stomata were more pronounced by exposure to the volatiles of these species than to mixtures of camphor and menthol, which influenced the growth of *Arabidopsis* leaves positively when applied in low amounts for short periods (Schulz *et al*. unpublished data). As expected, a similar reaction was observed in this study, although different volatile species were used. The results indicate that stomatal opening may be a more general effect when plants are exposed to monoterpenes or other related compounds.

However, according to Barros *et al*. (1997), internal water tension is unlikely to be an important factor controlling the coffee plant growth cycle. Despite these considerations, relatively little attention has been devoted to the understanding of vegetative growth behaviour during the growing season on a physiological basis related to environmental conditions. If volatile oils cause enlargement of stomatal apertures, growth may be stimulated, as a higher transpiration rate is to be expected. However, the important role of precipitation is to be considered too. Higher transpiration rates were observed with

Arabidopsis thaliana exposed to monoterpenes (Schulz *et al*. unpublished data). The superior growth rates observed under low altitude conditions in Orquidea Farm could be correlated with an increase in temperature and precipitation observed during the evaluation period for this experimental site. The localized effect on growth stimulation in *C. arabica* close to the intercropped herbs could be related to the important role of volatilization of monoterpens from herb oils into the environment and its effects on neighbour plants. Müller and Del Moral (1966) concluded that monoterpenes and other volatile compounds present in herbs can be absorbed at a greater rate during high temperature periods by surrounding plants during the dry season.

The relationships between vegetative and reproductive growth in coffee are rather complex and poorly understood. In most regions, rapid vegetative growth and fruit development appear to take place at different times. Nevertheless, the positive effect on appearance of new coffee leaves and an increase in branch length when intercropped with aromatic species is of considerable relevance because flower buds in *Arabica* coffee are initiated on the same wood only once (Rena *et al.,* 1994), thus the amount of growth produced in the current season will determine the crop yield of the following growing season. Therefore, additional studies have to be performed to compare the yield of coffee plants intercropped with aromatic species to that of control plants growing in the absence of aromatic plants.

5.3 Improvement of cup quality in coffee by volatiles of herbs

Another, unexpected effect of intercropped aromatic species was the improvement of the coffee cup quality. Sucrose is the principal sugar in coffee, and it acts as an aroma precursor, originating several substances (furans, aldehydes, carboxyl acids, etc.) which will affect both flavor and aroma of the beverage. Laboratory experiments revealed an increase on coffee stomata aperture due to exposure to volatiles from herbs. A potential correlation between the field results, where cup quality of coffee was better under treatments with herbs and laboratory results may be established. The herb volatiles, through their effect on stomata opening, may affect the photosynthetic activity of coffee plants, thereby increasing sucrose concentration in coffee beans, and positively affecting cup quality. The higher the sucrose contents in green beans, the more intense the coffee cup flavour.

Studies show that the quality of the beverage is especially connected to the altitude of the plots, the rainfall, the soil acidity, the percentage of shade, the yield of the trees, and the bean-size (Avelino, 2005). Results of this study shows that coffee quality was associated with altitude, with best coffees occurring at 900 m.a.s.l.

During roasting, coffee bean volume increases by half or more, but bean mass decreases by a fifth. Moreover, the number of volatile molecular species responsible for aroma formation increases from some 250 in green coffee, to more than 800 in roasted beans (Illy, 2002). Trigonelline is a pyridine derivative known to contribute indirectly to the formation of desirable aromas during roasting (Ky *et al*. 2001). Trigonelline is 100% soluble in water and therefore will end up in the cup. It is probably the most significant constituent contributing to excessive bitterness. However, association with exposure to volatiles from aromatic plants is obviously one of the agricultural factors that influence the composition of aroma precursors but the mechanism of action needs to be clarified.

5.4 Yield of intercropped herbs in coffee systems

There are two hypotheses about the role of the high concentrations of caffeine that accumulate in coffee production systems. Caffeine is a calcium release inducer that can result in a wide array of effects as calcium is used in plant cells for a number of purposes in membrane organization (Ashihara, 2006).

The chemical “defence theory” proposes that caffeine is present in young leaves, fruits and flower bud sacks to protect soft tissues from predators such as insect larvae and beetles. The “allelopathic theory” proposes that caffeine in seed coats is released into the soil and inhibits the germination of other seeds and development of plants to avoid competition.

In this study, data demonstrate that caffeine accumulated in the soil acts as a negative allochemical to aromatic plants when intercropped in coffee production systems, increasing coffee’s competitive advantage and supporting the second theory.

According to the available data, an estimation of production for the three different systems shows a decrease in yield with increasing age of the plot. Low altitude conditions are not suitable for sage, oregano, basil and mint. A possible ability of the aromatic plants to grow better under high altitude conditions may be associated with the temperature and water availability.

For coffee production systems between 5 and 10 years of establishment, an extra income of 400-500 kg of basil, sage and oregano can be obtained per cut. Three cuts during no harvest period can be done, obtaining 1500 (1500 or 1.5 kg?) kg/ ha^{-1} as extra income for coffee growers in crisis. The production of spearmint is not significant, and cannot compete with commercial production, but competition with weeds and coverage in between coffee rows open an interesting sustainable alternative for weed management in coffee production systems.

5.5 Modified atmospheres. A new alternative for herb's shelf life?

The shelf life of herbs is greatly affected by harvest time within the day. Generally, fresh herbs should be harvested early in the day, after any dew has evaporated but when crop turgidity is at its greatest (De Muth, 1996; Villamizar, 2001a). The analysis of commercialization opportunities indicates that modern facilities for processing and packaging are fundamental to open new and profitable markets.

The modified atmosphere (MA) enlarges the storage life of herbs and other vegetable products. Modified atmosphere is the reduction of the concentration of oxygen in air to levels below 1%, and posterior injection of a mixture of suitable gases into packaging materials, so as to have an atmosphere within the package varies over time in accordance with the needs and demands of the product. In our study, up to 100% of shelf life was increased by modifying the atmosphere during packing. However, no definite recommendations can be made based on this study alone, and further trials are need to determine the best possible atmosphere and package weight for each herb.

The factors that affect the intensity of these processes and the conditions of manipulation and commercialization must be taken into account when designing a packing system. A permeable polymeric film must be selected in order to use modified atmosphere for packing. The design of the package as well as its final weight and presentation are constrains to be analysed for each species of herbs produced. Demand is increasing for freshly harvested culinary herbs, but their extreme perishability and short shelf-life has resulted in high levels of wastage and a poor quality product which has limited the commercialisation and growth for local and export markets. It is necessary to evaluate new technologies to maintain shelving quality of the species for longer periods with a suitable

transport and presentation package. Overall, the main cause of deterioration during the evaluation period was leaf yellowing and blackening (necrosis of biomaterial). The results show damage to herbs can be reduced to less than 20% by packaging under modified atmospheres and vacuum packaging during the first week post-harvest. In general, all evaluated herbs benefited from atmosphere modification, in the second and third week of evaluation, increasing shelving life up to 100% in comparison with the traditional management.

5.6 Essential oils as growth stimulants in zucchini and beans

Some of the requirements of intensive agriculture make the use of plant growth regulators (PGRs) a feasible production tool for vegetables. Considering the economical importance of vegetable production, allelochemicals which stimulate their growth need to be identified and isolated for possible commercial use.

Results indicate that essential oils from basil, spearmint, oregano and sage strongly stimulate fruit weight in beans and zucchini under the ecological conditions and during the evaluated season.

The higher concentrations of oils increased the number of fruits in bean pods but reduce the fructification of zucchini, probably by repelling pollinator insects. Some reports from Riotte (1975) suggest that insect's pest damage to cabbage (*Brassica oleracea capitata* L.) can be reduced by intercropping rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.). They also suggest that some herbs might have some repelling effects over insects, reducing damage to the crops, but the mechanism is not clear.

According to Lundgren *et al*. (1978), the emission of volatiles from herbs provide a potential allelopathic "umbrella" effect, particularly in close proximity to surrounding plants. The higher average number of fruits in control zucchini plants could be the result of a repellent effect of the treatments applied on pollinator insects, resulting in a notable decrease in fructification for this species.

An increase in yield over that of control plants in both evaluated species due to the application of essential oils was observed. Based on the bioassays with *C. arabica* leaves,

stomata aperture was stimulated by essential oils. This effect could also be present in zucchini and beans leaves, increasing transpiration and photosynthetic activity and resulting in an increase in growth and fructification, thus water was not a limiting factor under the evaluated conditions.

6 CONCLUSIONS AND RECOMMENDATIONS

To summarize the results:

1. Spearmint and basil absorb caffeine and can diminish the caffeine contamination of coffee soils, if removed from the soil after uptake of the allelochemical. Further research needs to be conducted to identify and determine the absorption time and uptake quantity by these species under field conditions. As intercrops able to actively take-up caffeine, sage and oregano may help in retarding the early degeneration of coffee plantations by potentially metabolizing the alkaloid. This study indicates that caffeine absorption is not restricted to coffee plants; that other species such as herbs also absorb caffeine, though the mechanisms of absorption, release and possibly metabolization of caffeine need to be clarified in order to actively use these plants to detoxify and avoid early degeneration of coffee soils.

2. Intercropping sage, spearmint, basil and oregano stimulate the plagiotropic growth of *Coffea arabica* plants most effectively in young production systems. Furthermore, they induce stomatal opening in coffee leaves, which may have a positive influence on the CO_2 fixation when no other limiting factors are present and may increase the photosynthetic rates. The cup quality is improved principally with oregano and sage as intercrops, but mechanisms of action are unknown and further research on this topic needs to be done.

3. Coffee growers can stabilize their income and their social condition by offering aromatic plants produced during the no-harvest period of coffee to the local markets and using idle space of their farms. Therefore, additional studies have to be performed to compare yields of aromatic species under different coffee production systems. Proper post-harvest handling and packaging methods will help secure the supply of fresh quality herbs and will assist in capturing niche and developing markets for those who can supply sufficient quantities of quality product. In general, all evaluated herbs benefited from atmosphere modification, with shelf-life increasing up to 100% compared to shelf-life under traditional management.

4. Although there is a high potential of intercropping basil, sage, spearmint and oregano between coffees rows in order to obtain extra income for the idle area, evidence from this study indicates that age of the plots and accumulation of caffeine in the soil are limiting factors in the yield of aromatic plants. For coffee variables analyzed, branch length and the number of new coffee leaves formed were negatively influenced by age of plot and the possible accumulation of caffeine in older coffee soils.

5. A potential role of essential oils as growth stimulants on vegetables was observed for the evaluated conditions but further research needs to be conducted to determine additional advantages of using these compounds as growth stimulants in sustainable agriculture.

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8 ANNEX AND COMPLEMENTARY STUDIES

SOIL ANALYSES FROM THE EXPERIMENTAL PLOTS IN PUEBLA; MEXICO

ANNEX 1

ANNEX 2

ANNEX 3

ANNEX 4

ANNEX 5

ANNEX 6

ANNEX 7

STATISTICAL ANALYSIS

Appearance of new leaves in coffee according to treatments and age of the production systems

Analysis of Variance for new leaves - Type III Sums of Squares

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:treatments	2711,26	4	677,816	1,89	0,0096
B:plots	15263,9	5	3052,78	8,53	0,0000
INTERACTIONS					
AB	3650,62	20	182,531	0,51	0,9637
RESIDUAL	333029,0	930	358,095		
TOTAL (CORRECTED)	354654,0	959			

All F-ratios are based on the residual mean square error.

Multiple Range Tests for new leaves by treatments

Method: 95,0 percent LSD

treatments	Count	LS Mean	LS Sigma	Homogeneous Groups
control	192	22,8385	1,36568	X
oregano	192	24,8741	1,36568	XX
basil	192	26,2439	1,36568	XX
sage	192	26,3229	1,36568	XX
spearmint	192	27,8438	1,36568	X

Contrast	Difference	+/- Limits
basil - control	3,40538	3,78541
basil - oregano	1,36979	3,78541
basil - sage	-0,0789931	3,78541
basil - spearmint	-1,59983	3,78541
control - oregano	-2,03559	3,78541
control - sage	-3,48438	3,78541
control - spearmint	*-5,00521	3,78541
oregano - sage	-1,44878	3,78541
oregano - spearmint	-2,96962	3,78541
sage - spearmint	-1,52083	3,78541

* denotes a statistically significant difference.

Tukey test Number of new leaves by treatments

HBUENDIC

	TRATAMIE	N	Subset for alpha = .05	
			1	2
Tukey HSD[a]	control	96	24,31	
	albahaca	96	31,56	31,56
	oregano	96	31,83	31,83
	salvia	96	33,41	33,41
	menta	96		36,50
	Sig.		,147	,723
Duncan[a]	control	96	24,31	
	albahaca	96	31,56	31,56
	oregano	96	31,83	31,83
	salvia	96		33,41
	menta	96		36,50
	Sig.		,072	,261

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 96,000.

Tukey test number of new leaves by age of the production system

HBUENFEB

	LOTES	N	Subset for alpha = .05	
			1	2
Tukey HSD[a]	Lote viejo provi	160	13,29	
	Lote medio provi	160	15,14	15,14
	Lote joven provi	160		17,52
	Sig.		,521	,342
Duncan[a]	Lote viejo provi	160	13,29	
	Lote medio provi	160	15,14	15,14
	Lote joven provi	160		17,52
	Sig.		,276	,162

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

HBUEMAYO

	LOTES	N	Subset for alpha = .05		
			1	2	3
Tukey HSD[a]	Lote medio provi	160	15,76		
	Lote viejo provi	160	21,00		
	Lote joven provi	160		26,84	
	Sig.		,051	1,000	
Duncan[a]	Lote medio provi	160	15,76		
	Lote viejo provi	160		21,00	
	Lote joven provi	160			26,84
	Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

HBUENJUN

	LOTES	N	Subset for alpha = .05		
			1	2	3
Tukey HSD[a]	Lote medio provi	160	19,85		
	Lote viejo provi	160		28,29	
	Lote joven provi	160		33,83	
	Sig.		1,000	,103	
Duncan[a]	Lote medio provi	160	19,85		
	Lote viejo provi	160		28,29	
	Lote joven provi	160			33,83
	Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

HBUENOCT

	LOTES	N	Subset for alpha = .05	
			1	2
Tukey HSD[a]	Lote medio provi	160	32,51	
	Lote viejo provi	160	39,74	39,74
	Lote joven provi	160		45,54
	Sig.		,154	,299
Duncan[a]	Lote medio provi	160	32,51	
	Lote viejo provi	160	39,74	39,74
	Lote joven provi	160		45,54
	Sig.		,065	,138

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

HBUENDIC

	LOTES	N	Subset for alpha = .05	
			1	2
Tukey HSD[a]	Lote medio provi	160	27,15	
	Lote viejo provi	160	30,19	30,19
	Lote joven provi	160		37,23
	Sig.		,581	,056
Duncan[a]	Lote medio provi	160	27,15	
	Lote viejo provi	160	30,19	
	Lote joven provi	160		37,23
	Sig.		,320	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

Table of Means for new leaves

with 95,0 Percent Confidence Intervals

Level		Stnd. Count	Mean	Error	Lower Limit	Upper Limit
GRAND MEAN		960	25,6247			
treatments						
basil		192	26,2439	1,36568	23,5672	28,9206
control		192	22,8385	1,36568	20,1619	25,5152
oregano		192	24,8741	1,36568	22,1974	27,5508
sage		192	26,3229	1,36568	23,6462	28,9996
spearmint		192	27,8438	1,36568	25,1671	30,5204
plots						
medium orq		160	22,325	1,49603	19,3928	25,2572
medium prov		160	25,7927	1,49603	22,8605	28,7249
new orq		160	26,1875	1,49603	23,2553	29,1197
new prov		160	33,8729	1,49603	30,9408	36,8051
old orq		160	22,7385	1,49603	19,8064	25,6707
old prov		160	22,8312	1,49603	19,8991	25,7634
treatments by plots						
basil	medium orq	32	23,2031	3,34522	16,6466	29,7596
basil	medium pro	32	26,4323	3,34522	19,8758	32,9888
basil	new orq	32	26,4219	3,34522	19,8654	32,9784
basil	new prov	32	34,8021	3,34522	28,2456	41,3586
basil	old orq	32	25,7969	3,34522	19,2404	32,3534
basil	old prov	32	20,8073	3,34522	14,2508	27,3638
control	medium orq	32	20,8646	3,34522	14,3081	27,4211
control	medium pro	32	22,1875	3,34522	15,631	28,744
control	new orq	32	24,1823	3,34522	17,6258	30,7388
control	new prov	32	31,7083	3,34522	25,1518	38,2649
control	old orq	32	17,3698	3,34522	10,8133	23,9263
control	old prov	32	20,7188	3,34522	14,1622	27,2753
oregano	medium orq	32	23,0	3,34522	16,4435	29,5565
oregano	medium pro	32	22,5677	3,34522	16,0112	29,1242
oregano	new orq	32	25,6875	3,34522	19,131	32,244
oregano	new prov	32	30,0833	3,34522	23,5268	36,6399
oregano	old orq	32	22,5833	3,34522	16,0268	29,1399
oregano	old prov	32	25,3229	3,34522	18,7664	31,8794
sage	medium orq	32	23,8542	3,34522	17,2976	30,4107
sage	medium pro	32	27,1302	3,34522	20,5737	33,6867
sage	new orq	32	23,1875	3,34522	16,631	29,744
sage	new prov	32	36,5104	3,34522	29,9539	43,0669
sage	old orq	32	22,1927	3,34522	15,6362	28,7492
sage	old prov	32	25,0625	3,34522	18,506	31,619
spearmint	medium orq	32	20,7031	3,34522	14,1466	27,2596
spearmint	medium pro	32	30,6458	3,34522	24,0893	37,2024
spearmint	new orq	32	31,4583	3,34522	24,9018	38,0149
spearmint	new prov	32	36,2604	3,34522	29,7039	42,8169
spearmint	old orq	32	25,75	3,34522	19,1935	32,3065
spearmint	old prov	32	22,2448	3,34522	15,6883	28,8013

Branch length in coffee according to treatments and age of the production systems

Analysis of Variance for branch growth –
Type III Sums of Squares

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
MAIN EFFECTS					
A:treatments	18,4104	4	4,6026	0,09	0,9856
B:plots	8285,88	5	1657,18	32,37	0,0000
INTERACTIONS					
AB	1735,13	20	86,7564	1,69	0,0289
RESIDUAL	47604,9	930	51,188		
TOTAL (CORRECTED)	57644,3	959			

All F-ratios are based on the residual mean square error.

Multiple Range Tests for branch growth by treatments

Method: 95,0 percent LSD

treatments	Count	LS Mean	LS Sigma	Homogeneous Groups
sage	192	16,8229	0,516337	X
oregano	192	17,1146	0,516337	X
spearmint	192	17,1302	0,516337	X
control	192	17,1562	0,516337	X
basil	192	17,224	0,516337	X

Contrast	Difference	+/- Limits
basil - control	0,0677083	1,43119
basil - oregano	0,109375	1,43119
basil - sage	0,401042	1,43119
basil - spearmint	0,09375	1,43119
control - oregano	0,0416667	1,43119
control - sage	0,333333	1,43119
control - spearmint	0,0260417	1,43119
oregano - sage	0,291667	1,43119
oregano - spearmint	-0,015625	1,43119
sage - spearmint	-0,307292	1,43119

* denotes a statistically significant difference.

Tukey test number of branch growth by age of the production system

LONGFEBR

	LOTES	N	Subset for alpha = .05	
			1	2
Tukey HSD[a]	Lote medio provi	160	50,71	
	lote medio orqui	160	52,51	
	Lote viejo provi	160	52,63	
	lote joven orqui	160	53,26	
	lote viejo orqui	160	53,31	
	Lote joven provi	160	53,98	
	Sig.		,184	
Duncan[a]	Lote medio provi	160	50,71	
	lote medio orqui	160	52,51	52,51
	Lote viejo provi	160	52,63	52,63
	lote joven orqui	160	53,26	53,26
	lote viejo orqui	160	53,31	53,31
	Lote joven provi	160		53,98
	Sig.		,100	,362

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

LONGMAYO

	LOTES	N	Subset for alpha = .05		
			1	2	3
Tukey HSD[a]	Lote medio provi	160	53,51		
	Lote viejo provi	160	55,34	55,34	
	lote medio orqui	160	56,44	56,44	
	lote viejo orqui	160	56,61	56,61	
	Lote joven provi	160	57,31	57,31	
	lote joven orqui	160		58,68	
	Sig.		,079	,175	
Duncan[a]	Lote medio provi	160	53,51		
	Lote viejo provi	160	55,34	55,34	
	lote medio orqui	160		56,44	56,44
	lote viejo orqui	160		56,61	56,61
	Lote joven provi	160		57,31	57,31
	lote joven orqui	160			58,68
	Sig.		,195	,210	,154

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

LONGJUN

	LOTES	N	Subset for alpha = .05		
			1	2	3
Tukey HSD[a]	Lote medio provi	160	58,08		
	Lote viejo provi	160	59,57	59,57	
	lote medio orqui	160	61,49	61,49	61,49
	Lote joven provi	160	62,11	62,11	62,11
	lote viejo orqui	160		62,27	62,27
	lote joven orqui	160			63,88
	Sig.		,061	,425	,566
Duncan[a]	Lote medio provi	160	58,08		
	Lote viejo provi	160	59,57	59,57	
	lote medio orqui	160		61,49	61,49
	Lote joven provi	160		62,11	62,11
	lote viejo orqui	160		62,27	62,27
	lote joven orqui	160			63,88
	Sig.		,305	,089	,135

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

LONGAGOS

			Subset for alpha = .05		
	LOTES	N	1	2	3
Tukey HSD[a]	Lote medio provi	160	60,04		
	Lote viejo provi	160	61,38	61,38	
	lote medio orqui	160	63,63	63,63	63,63
	Lote joven provi	160	63,94	63,94	63,94
	lote viejo orqui	160		64,51	64,51
	lote joven orqui	160			66,29
	Sig.		,077	,254	,438
Duncan[a]	Lote medio provi	160	60,04		
	Lote viejo provi	160	61,38	61,38	
	lote medio orqui	160		63,63	63,63
	Lote joven provi	160		63,94	63,94
	lote viejo orqui	160			64,51
	lote joven orqui	160			66,29
	Sig.		,355	,095	,093

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

LONGOCT

			Subset for alpha = .05			
	LOTES	N	1	2	3	4
Tukey HSD[a]	Lote medio provi	160	63,49			
	Lote viejo provi	160	64,96	64,96		
	lote medio orqui	160	67,51	67,51	67,51	
	Lote joven provi	160		68,49	68,49	
	lote viejo orqui	160		68,59	68,59	
	lote joven orqui	160			70,76	
	Sig.		,080	,148	,253	
Duncan[a]	Lote medio provi	160	63,49			
	Lote viejo provi	160	64,96	64,96		
	lote medio orqui	160		67,51	67,51	
	Lote joven provi	160			68,49	68,49
	lote viejo orqui	160			68,59	68,59
	lote joven orqui	160				70,76
	Sig.		,327	,089	,498	,155

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

LONGDIC

			Subset for alpha = .05			
	LOTES	N	1	2	3	4
Tukey HSD[a]	Lote medio provi	160	64,43			
	Lote viejo provi	160	65,91	65,91		
	Lote joven provi	160		69,60	69,60	
	lote medio orqui	160			71,22	71,22
	lote viejo orqui	160			72,62	72,62
	lote joven orqui	160				74,74
	Sig.		,929	,158	,365	,200
Duncan[a]	Lote medio provi	160	64,43			
	Lote viejo provi	160	65,91			
	Lote joven provi	160		69,60		
	lote medio orqui	160		71,22		
	lote viejo orqui	160		72,62	72,62	
	lote joven orqui	160			74,74	
	Sig.		,336	,063	,169	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 160,000.

Table of Means for branch growth
with 95,0 Percent Confidence Intervals

Level		Count	Mean	Stnd. Error	Lower Limit	Upper Limit
GRAND MEAN		960	17,0896			
treatments						
basil		192	17,224	0,516337	16,212	18,236
control		192	17,1562	0,516337	16,1442	18,1683
oregano		192	17,1146	0,516337	16,1026	18,1266
sage		192	16,8229	0,516337	15,8109	17,8349
spearmint		192	17,1302	0,516337	16,1182	18,1422
plots						
medium orq		160	19,3063	0,565619	18,1977	20,4148
medium prov		160	13,7187	0,565619	12,6102	14,8273
new orq		160	21,4813	0,565619	20,3727	22,5898
new prov		160	15,6187	0,565619	14,5102	16,7273
old orq		160	18,7063	0,565619	17,5977	19,8148
old prov		160	13,7062	0,565619	12,5977	14,8148
treatments by plots						
basil	medium orq	32	20,0938	1,26476	17,6149	22,5726
basil	medium pro	32	14,8437	1,26476	12,3649	17,3226
basil	new orq	32	20,0313	1,26476	17,5524	22,5101
basil	new prov	32	16,4687	1,26476	13,9899	18,9476
basil	old orq	32	19,0625	1,26476	16,5836	21,5414
basil	old prov	32	12,8437	1,26476	10,3649	15,3226
control	medium orq	32	21,1875	1,26476	18,7086	23,6664
control	medium pro	32	12,5625	1,26476	10,0836	15,0414
control	new orq	32	22,9688	1,26476	20,4899	25,4476
control	new prov	32	15,4063	1,26476	12,9274	17,8851
control	old orq	32	18,5313	1,26476	16,0524	21,0101
control	old prov	32	12,2812	1,26476	9,80235	14,7601
oregano	medium orq	32	19,5313	1,26476	17,0524	22,0101
oregano	medium pro	32	12,0937	1,26476	9,61485	14,5726
oregano	new orq	32	19,7188	1,26476	17,2399	22,1976
oregano	new prov	32	14,6563	1,26476	12,1774	17,1351
oregano	old orq	32	19,8438	1,26476	17,3649	22,3226
oregano	old prov	32	16,8437	1,26476	14,3649	19,3226
sage	medium orq	32	16,875	1,26476	14,3961	19,3539
sage	medium pro	32	15,5937	1,26476	13,1149	18,0726
sage	new orq	32	22,4375	1,26476	19,9586	24,9164
sage	new prov	32	16,5	1,26476	14,0211	18,9789
sage	old orq	32	18,3125	1,26476	15,8336	20,7914
sage	old prov	32	11,2187	1,26476	8,73985	13,6976
spearmint	medium orq	32	18,8438	1,26476	16,3649	21,3226
spearmint	medium pro	32	13,5	1,26476	11,0211	15,9789
spearmint	new orq	32	22,25	1,26476	19,7711	24,7289
spearmint	new prov	32	15,0625	1,26476	12,5836	17,5414
spearmint	old orq	32	17,7813	1,26476	15,3024	20,2601
spearmint	old prov	32	15,3437	1,26476	12,8649	17,8226

Harvest of aromatic herbs according to species and age of the production systems

Analysis by age of the production system

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
COS1GR	Between Groups	41107,292	2	20553,646	,454	,636
	Within Groups	8564877	189	45316,809		
	Total	8605984	191			
COS2GR	Between Groups	3834495	2	1917247,266	7,003	,001
	Within Groups	51743551	189	273775,401		
	Total	55578045	191			

COS1GR

			Subset for alpha = .05
	LOTES	N	1
Tukey HSD[a]	viejo	64	264,92
	medio	64	281,02
	nuevo	64	300,70
	Sig.		,609
Duncan[a]	viejo	64	264,92
	medio	64	281,02
	nuevo	64	300,70
	Sig.		,375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 64,000.

COS2GR

			Subset for alpha = .05	
	LOTES	N	1	2
Tukey HSD[a]	viejo	64	499,53	
	medio	64		773,98
	nuevo	64		819,45
	Sig.		1,000	,875
Duncan[a]	viejo	64	499,53	
	medio	64		773,98
	nuevo	64		819,45
	Sig.		1,000	,624

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 64,000.

Analysis by specie

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
COS1GR	Between Groups	625043,1	3	208347,700	4,908	,003
	Within Groups	7980941	188	42451,815		
	Total	8605984	191			
COS2GR	Between Groups	14561322	3	4853774,132	22,247	,000
	Within Groups	41016723	188	218174,058		
	Total	55578045	191			

COS1GR

			Subset for alpha = .05	
	TRAT	N	1	2
Tukey HSD[a]	menta	48	193,02	
	oregano	48	271,88	271,88
	salvia	48		330,21
	albahaca	48		333,75
	Sig.		,242	,457
Duncan[a]	menta	48	193,02	
	oregano	48	271,88	271,88
	salvia	48		330,21
	albahaca	48		333,75
	Sig.		,062	,168

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 48,000.

COS2GR

			Subset for alpha = .05		
	TRAT	N	1	2	3
Tukey HSD[a]	oregano	48	425,73		
	menta	48	451,77		
	albahaca	48		824,48	
	salvia	48			1088,65
	Sig.		,993	1,000	1,000
Duncan[a]	oregano	48	425,73		
	menta	48	451,77		
	albahaca	48		824,48	
	salvia	48			1088,65
	Sig.		,785	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 48,000.

Analysis by plot –specie interaction

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
COS1GR	Between Groups	3645124	5	729024,818	27,334	,000
	Within Groups	4960860	186	26671,291		
	Total	8605984	191			
COS2GR	Between Groups	9195459	5	1839091,875	7,375	,000
	Within Groups	46382586	186	249368,742		
	Total	55578045	191			

COS1GR

	LOTES	N	Subset for alpha = .05			
			1	2	3	4
Tukey HSD[a]	medio orqui	32	102,34			
	joven orqui	32	147,19	147,19		
	viejo orqui	32		236,25	236,25	
	viejo provi	32			293,59	
	joven provi	32				454,22
	medio provi	32				459,69
	Sig.		,882	,251	,724	1,000
Duncan[a]	medio orqui	32	102,34			
	joven orqui	32	147,19			
	viejo orqui	32		236,25		
	viejo provi	32		293,59		
	joven provi	32			454,22	
	medio provi	32			459,69	
	Sig.		,273	,162	,894	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 32,000.

COS2GR

	LOTES	N	Subset for alpha = .05	
			1	2
Tukey HSD[a]	viejo orqui	32	437,81	
	medio orqui	32	545,31	
	viejo provi	32	561,25	
	joven provi	32	653,13	653,13
	joven orqui	32		985,78
	medio provi	32		1002,66
	Sig.		,517	,062
Duncan[a]	viejo orqui	32	437,81	
	medio orqui	32	545,31	
	viejo provi	32	561,25	
	joven provi	32	653,13	
	joven orqui	32		985,78
	medio provi	32		1002,66
	Sig.		,118	,893

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 32,000.

ERKLÄRUNG

Ich versichere, dass ich diese Arbeit selbständig verfasst habe, keine anderen quellen und Hilfsmittel als die angegebenen benutzt und die Stellen der Arbeit, die anderen Werken dem Wortlaut oder dem Sinn nach entnommen sind, kenntlich gemacht habe.

Die Arbeit hat in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegen.

Bonn, den 26 März 2007

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Alex Gustavo Pacheco Bustos