Biofumigation

Concept note & compilation of session guides on Biofumigation

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Inter-country Programme for Vegetable IPM in South & SE Asia
Phase II
(FNPP/GLO/002/NET & GCP/RAS/191/AUL)

Food & Agriculture Organization of the United Nations
March 2005

Foreword

The Biofumigation concept is being introduced to Asian vegetable smallholders under the auspices of the FAO Regional Vegetable IPM Programme Phase II. Being a relatively new ecologically, socially and economically viable option against a plethora of persistent and ever increasing soil-borne disease problems, it requires localization, experience and adoption with the potential to develop a unique biocontrol option. However, it also requires attention from the scientific communities to further refine the application methods, identifying better-suited varieties of Brassicaceous crops high in glucosinolates to suit farmers producing under various socio-economic conditions. After phasing out of Methyl Bromide, Biofumigation has gained increasing attention as an option in the sustainable management of soil borne-pathogens, nematodes and to some extent bacterial diseases originating from the soil.

Sincere thanks goes to Dr John Kirkegaard based at CSIRO in Canberra, Australia and Ms. Vale Justo, Entomologist at the National Crop Protection Center in Los Banos, the Philippines. Both have allowed me to report on the most updated biofumigation research findings resulting from an ongoing ACIAR research project. I would like to express sincere thanks to Dr. Kirkegaard for reading the document and proving valuable inputs.

Acknowledgements also go to Dr. Maxwell J. Whitten (former Chief Technical Advisor of the FAO Regional Vegetable IPM Program) for bringing this concept from the premises of the Australian laboratory to the knowledge domain and vegetable fields of Asia. Max not only brought the concept here, but also he kept us all updated on the scientific advances made in this area from time to time. In addition, to all other colleagues, who provided/shared information related to Biofumigation to be used in Asian farms. A special vote of thanks goes to the Team Leader, Mr. Jan Willem Ketelaar for actively advancing the concept and practice through TOT/FFS, farmer's research in the region in the member countries of the FAO Regional Vegetable IPM Programme Phase II

Sincere thanks goes to the National Vegetable IPM programs and its trainers in Cambodia, China and Vietnam for turning this concept into a new option for disease and insect-pest management. Special thanks goes to Ms. Abha Mishra, PhD student, Agriculture Systems and Engineering Field of Study, School of Environment Resources and Development, Asian Institute of Technology (AIT), Bangkok for helping with preparation of the technical introduction on Biofumigation, and other scientific background information used in this document.

Finally, I hope that this document would serve as ready and handy reference and source of inspiration for trainers and farmers in the region and elsewhere to actively use Biofumigation as economic and sustainable alternative for management of soil borne pests.

KUMAR, Prabhat
March 2005

*cover page picture is taken at the 2003 Training of Trainers Course, Siem-Reap, Cambodia – Trainers are grinding different sources of local Brassicaceous crops before field application. Courtesy: National IPM Program, Cambodia. *Recent research findings from the ACIAR Biofumigation project suggest that such grinding methods would NOT provide for the necessary levels of macerations at the tissue level and thus provide for insufficient release of the biocidal compounds of glucosinolates and isothiocyanates.*

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1. Background

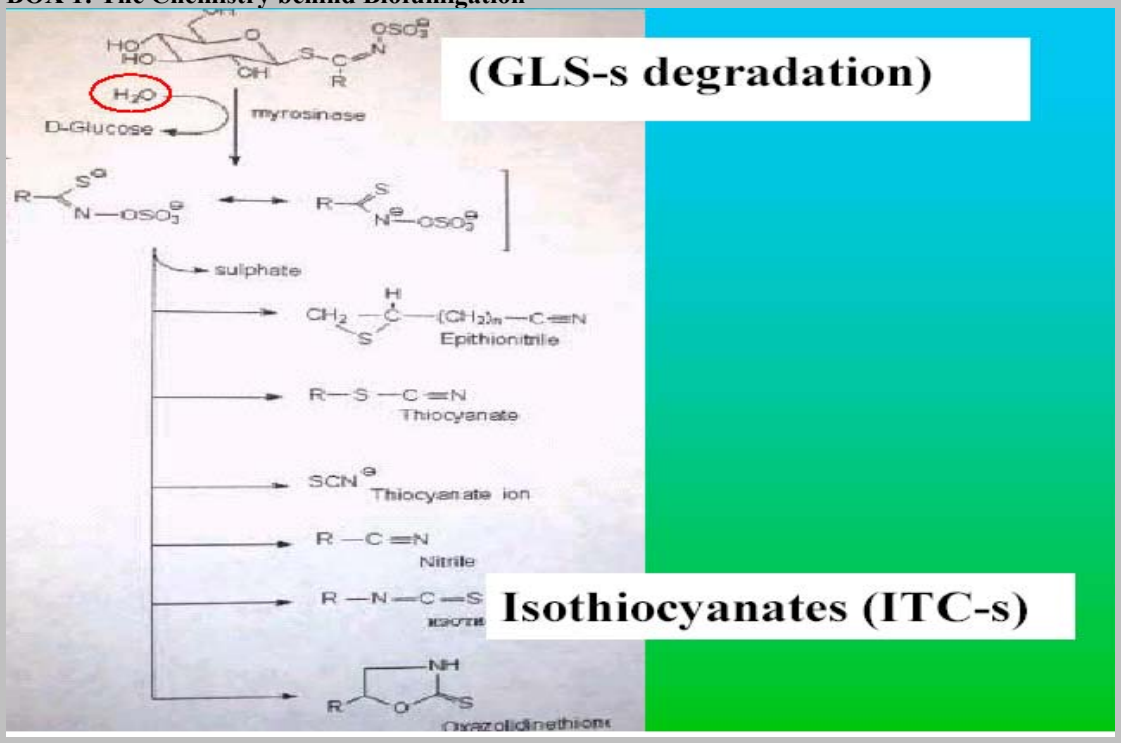
Managing soil-borne pests and diseases is critical for smallholder vegetable producers. Vegetables crops are vulnerable to a range of pathogenic organisms that reduce yield by killing the plant or damage the product and make it unmarketable. Insects, nematodes, fungi and bacteria are among the pests attacking the roots of vegetable crops. Because these pests are hidden in the soil, they are difficult to diagnose or find. This means that the threshold-based intervention methods used in integrated pest management (IPM) programs for vegetable crops can not be used for controlling root pests and pathogens. Control of soil borne pathogens is complicated because even very low populations are often highly damaging, the organisms are hidden and unevenly distributed in the soil, and are often microscopic in size. Farmers therefore often resort to prophylactic application of pesticides prior to planting as insurance against the crop damage and yield loss. It is usually too late and impractical to apply pesticides for control of soil-borne pest organisms during crop growth effectively. Horticulture is faced with several issues when controlling subterranean pests and diseases. Residual pesticides can no longer be used for control of soil insects; methyl bromide has been phased out under *The Montreal Protocol on Substances that Deplete the Ozone Layer*, to which most countries are signatories, whereas the demand for blemish-free produce is increasing. This is one of the reasons why Biofumigation is being investigated to control soil borne diseases with the aim to develop bio-pesticides, which could be effective against various root pathogens without degrading the soil environment.

2. What is Biofumigation?

Biofumigation is the agronomic practice of using volatile chemicals (allelochemicals), released from decomposing *Brassica* tissues, to suppress soil-borne pests and pathogens. The most common volatiles produced during the breakdown of Brassicas are isothiocyanates (ITCs). ITCs are related to the active ingredient in the commercial fumigant metham sodium and dazomet and are highly toxic to pests and pathogens. They are released following tissue damage, when myrosinase enzymes, at neutral pH, hydrolyse (in presence of water) glucosinolates (GSLs). GSLs are sulfur-containing chemicals (thioglucosides) that are produced as secondary metabolites by Brassicas and most researchers believe their evolutionary role is to provide resistance against pests and pathogens.

The biocidal activity of various isothiocyanates (ITCs) released by *Brassica* tissues is well-known (Brown and Morra 1997) and the potential of Brassicas to suppress a range of soil-borne pests and diseases is supported by considerable empirical field evidence (Matthiessen and Kirkegaard 1998). The chemical conversions of GSLs to ITCs are enumerated in *Box -1*.

BOX 1: The Chemistry behind Biofumigation



Most of us experience the phenomenon of Biofumigation in one way or another in our daily life, not always through growing crops but rather through exposure to exotic cuisines (*Box 2*).

3. Key points of Biofumigation

Biofumigation may:

- ⇒ Reduce weed competition and soil-borne pathogens;
- ⇒ Change the composition of soil populations of nematodes, bacteria, and post harvest pathogenic fungi;
- ⇒ Hold promise as a management tool for conventional and organic farmers, who are currently under pressure to find alternatives to chemical fumigation practices, and more ecologically acceptable means of reducing soil pathogens to improve or maintain yields;
- ⇒ Alter below ground ecology resulting in different rates of nutrient uptake by plants;
- ⇒ Improve soil physical structure by increasing soil porosity if used as green manure;

⇒ Add more organic carbon to the soil which is needed to increase the activity of functional groups (flora and fauna) of the soil;
⇒ Target a range of pathogens (broad spectrum) but do not persist in the soil for long periods because of the high reactivity of ITCs.

Box 2: Some common daily life kitchen experiences of Bio-fumigation

The Wasabi (*Wasabia japonica* ; *Wasabia tenuis*) : Wasabi paste, which is a main condiment used with Japanese cuisine " Sushi" is made of from plant materials of a member of the Cruciferae or mustard family, the wasabi plant; and is an evergreen crucifer that grows naturally in wet, cool mountain river valleys along stream beds and on river sand bars in Japan. Hydration of a glucoside (sinigrin) by the enzyme myrosinase results in the production of Wasabi's special flavor component, an allyl isothiocyanate, the major pungent component. We all experience this in the form of that heat rush up our noses when eating Wasabi with our sushi! The components that give Wasabi its flavor are 6-methylthiohexyl isothiocyanate, 7-methylthioheptyl isothiocyanate and 8-methylthioocytl isothiocyanate (Ina et al., 1989). Scientists are now discovering that these Wasabi isothiocyanates may have important medical benefits. Today research is being conducted both in the United States and in Japan as to the potential medical benefit of Wasabi. Researchers say that the isothiocyanates in Wasabi, not only inhibit microbes, but can also help treat or prevent blood clotting, asthma and even cancer. (J. A. Depree, T.M. Howard & G.P. Savage, Food Research International Vol 31, No5, pp.329-337, 1999). Wasabi has even been known to prevent tooth decay. (Hideki Masuda, Ph.D. 2000).
(Source: http://www.freshwasabi.com/wasabi_technical.htm)

Garden/pepper cress (*Lepidium sativum*)**:** *Lepidium sativum*, also known as garden cress, curled grass or pepper grass is a grass like plant that originated in the Eastern Mediterranean region. It is a member of cruciferous family, including kale, broccoli, and cabbage. Cress contains a volatile, sulphuretted oil, and myrosin. When you chew it, it gives you taste of mustard. These substances stimulate the metabolism and kidney activity, strengthen the stomach and gallbladder. Cress is also believed to have a curative effect on joint disorders and gout. Cress is also an important source of calcium, iron, vitamin A and vitamins C and E, which are two antioxidants nutrients that help protect the cells from damage by free radicals. (Source:http://ks.essortment.com/naturalhomesre_rmss.htm)

Mustard **(***Brassica alba, B. juncea, B. nigra; syn: Sinapsis* alba; Fam: Cruciferae): It was the condiment, not the plant, that was originally called mustard. The condiment was given its name because it was made by grinding the seeds of what was once called the senvy plant into a paste and mixing it with must (an unfermented wine). Mustard is one of the oldest spices and one of the most widely used. The Chinese were already using mustard thousands of years ago and the ancient Greeks considered it an everyday spice. The first medical mention of it is in the Hippocratic writings, where it was used for general muscular relief. The Romans used it as a condiment and pickling spice. King Louis XI would travel with his own royal mustard pot, in case his hosts didn't serve it. Today, world consumption of mustard tops 400 million pounds/year.

Whole white mustard seed is used in pickling spice and in spice mixtures for cooking meats and seafood. It adds piquancy to Sauerkraut and is sometimes used in marinades. In India, whole seeds are fried in ghee until the seed pops, producing a milder nutty flavour that is useful as a garnish or seasoning for other Indian dishes. The brown seed is also pounded with other spices in the preparation of curry powders and pastes. Mustard oil is made from *B. juncea*, providing a piquant oil widely used in India in the same way as ghee. Powdered mustard acts as an emulsifier in the preparation of mayonnaise and salad dressings. Powdered mustard is also useful for flavouring barbecue sauces, baked beans, many meat dishes, deviled eggs, beets and succotash. There are many ready-made mustards from mild and sweet to sharp and strong. They can be smooth or coarse and flavored with a wide variety of herbs, spices and liquids. In China and SE Asia, numerous species of leaf mustard are grown and consumed as vegetables. Apart from these uses, mustard has many medicinal properties and oil-cakes are used as soil amendments to control nematodes and soil diseases in many parts of South Asia.
(http://www.theepicentre.com/Spices/mustard.html)

4. Brassica / Brassica products available for Biofumigation

The following Brassica plant parts and/or derivatives have Biofumigation properties:

- ⇒ Brassica plants as cover crops or intercrops can be slashed and ploughed under at flowering stage to achieve maximum benefit;
- ⇒ Brassica material collected from the field or from markets can be macerated to form a "brew" which could be applied as a spot soil drench to soil pre-plant;
- ⇒ Brassica seed meal after crushing for oil used as a cake or powder which can be incorporated into the soil and may be used as mulch;
- ⇒ Volatile oil of Mustard (VOOM), which is the pure ITC extracted from mustard prepared as an emulsion in canola oil and can be used as pre-planting application as an alternative to methyl bromide.

Box 3: Summary of the suppressive impacts of incorporated *Brassica* green manures on a range of pest organisms*.

	Pest	*Brassica used*	*Suppression*	*Reference*
Suppression	Soldier fly	Kale, radish	76 - 86%	Blank *et al.* (1982)
	Bacterial wilt	Indian mustard	40 - 80%	Akiew *et al.* (1999)
	Meloidogyne (root knot nematode)	rapeseed	53 - 78%	McLeod and Steele (1999) Mojtahedi *et al.* (1993
	Aphanomyces	white mustard	29 - 54%	Muehlchen *et al.* (1990) Chan and Close (1987)
	Weeds	rapeseed	50 - 96%	Boydston and Hang (1995)
	Verticillium dahliae	rapeseed	0 %	Davis *et al.* (1996)
No suppression	*Rhizoctonia Pythium Meloidogyne*	rapeseed	0 %	Johnson *et al.* (1992)
Pathogen stimulation	*Pythium*	Canola, mustard	+ 8%	Stephens *et al.* (1999)

* Taken from Kirkegaard, J. and Matthiessen, J., 2004-5. Developing and refining the Biofumigaiton concept. Agroindustria / Vol. 3 / Num. 3 2004 5

5. Current status of Biofumigation Research

5.1 Biofumigation: mode of action, plant source and target organisms

Both scientific studies and general observations have shown that various Brassicas can produce suppressive effects on soil pests and diseases. The effects are related to the ITCs that form from precursor glucosinolates (GSLs) when the plant is disrupted, such as when it is incorporated into soil. There are many different GSLs in Brassicas, with about six types being most common. The toxicity of an ITC to various organisms can differ suggesting that specific plants could be utilized more successfully than others for biofumigant effects by matching them to particular pests or diseases. Aromatic ITCs produced from GSLs often found in roots are very toxic (50 or more times greater than metham sodium's MITC) but as they are of low volatility and appear to be more inactivated in soil so that contact with organisms may be reduced. Aliphatic ITCs are more common in shoots, and while less toxic, their greater volatility and lower sorption in soil may allow for greater contact with soil organisms.

Roots may release ITCs during growth as well as during decomposition. Consequently, the Biofumigation potential of roots should not be overlooked.

Biofumigation research has generated the following research and focus points:

- ⇒ ITC is the main active ingredient for the suppressive effect;
- ⇒ ITCs exist in both roots and shoots;
- ⇒ Type and concentration of ITC varies with species, variety, plant part as well as management (e.g. more sulphur = more glucosinolates = more ITC);
- ⇒ Incorporation of Brassica green manure in soil has shown suppressive impact on a range of pest and disease organisms (*see Box 3 above*);
- ⇒ Bacteria are generally less susceptible to ITCs than fungi;
- ⇒ No residual effect as ITC is highly volatile and reactive.

5.2 Findings from the ACIAR Project in the Philippines[1]

5.2.1 Background and objectives of the project

An ongoing bilateral research project on biofumigation project is funded by ACIAR involving the National Crop Protection Center (NCPC) in Los Banos, the Philippines and QDPI Mareeba and CSIRO Plant Industry Canberra, Australia. This project, titled "Evaluating Biofumigation for Soil-Borne Diseases Management in Tropical Vegetable Production" was initiated in 2001 with following major objectives:

[1] This section is taken from the ACIAR Review Report LWR2/2000/114, Evaluating Biofumigaiton for Soil-Borne Diseases Management in Tropical Vegetable Production. Compiled by Dr JA Kirkegaard, Project Leader; 19-21 May Mareeba, 2004. (john.kirkegaard@csiro.au)

- Identify candidate brassicas suitable for use as biofumigants in tropical vegetable production systems in the Philippines and northern Queensland, Australia;
- Evaluate the suppressive potential of candidate *Brassica* species, varieties and their various plant parts;
- Identify the biocidal compounds responsible for suppression and demonstrate a correlation between concentrations of these in the tissues and the degree of pest suppression;
- Identify factors which influence the efficiency of biocide release from the incorporated *Brassica* tissues;
- Evaluate the most promising *Brassica* species/varieties in the field and develop protocols for the most effective strategies to improve field efficacy based on the above;
- Evaluate effectiveness in commercial and small-scale farmer fields as a component of integrated disease management based on combined strategies including disease resistance/tolerance, non-host crop rotation and solarisation;
- Extend the existing Biofumigation Network (~700 recipients) to include a research and extension network in south-east Asia and the Pacific region to facilitate the development and transfer of this new technology.

5.2.2 Key findings of the project

Below –and in *Box 4*- are listed some key findings of the ACIAR biofumigation project to date:

- ***Tissue maceration-*** The work has demonstrated that volatile compounds released from *Brassica* tissues (such as ITCs) are very effective in suppression of both Bacterial Wilt and Root Knot Nematodes in laboratory and glasshouse experiments, but this only becomes evident when experimental conditions are conducive to ITC release. These ***conditions include significant tissue maceration at a cellular level to facilitate enzymatic hydrolysis and release of the ITCs, adequate water to facilitate hydrolysis, and containment to reduce volatile losses.*** Where the tissues were chopped rather than macerated, where soil was often relatively dry, where tissues were not mixed throughout the soil or where volatile loss was likely, there was rarely any clear link between GSL types or concentration and pathogen suppression as ITCs were not released or retained effectively.

- ***Brassica Green Manure –*** The study has served to highlight the mechanisms of pest suppression from incorporated green manures, and other resulting benefits to crops, as these may arise from a range of mechanisms as summarized below in *Figure 3*.

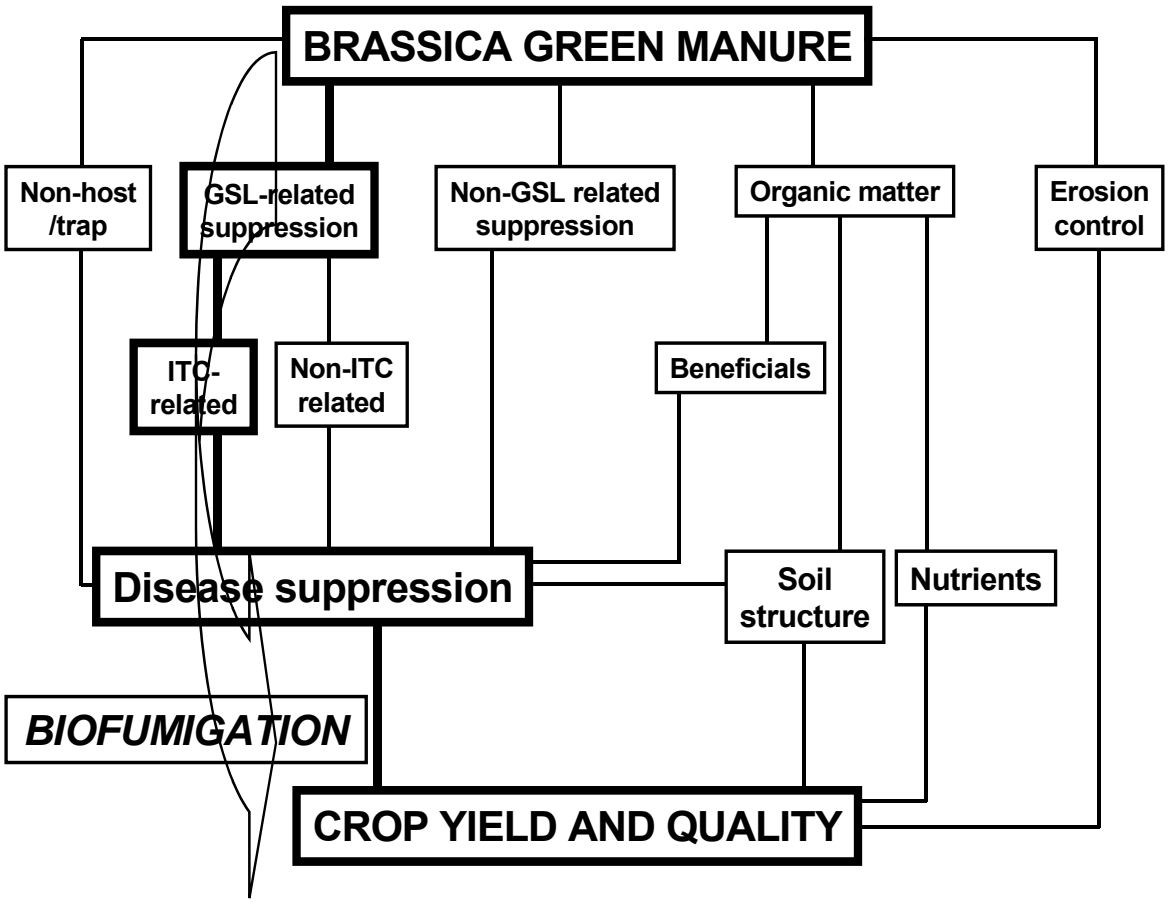


Figure 3: is taken from the ACIAR Review Report LWR2/2000/114, Evaluating Biofumigaiton for Soil-Borne Diseases Management in Tropical Vegetable Production. Compiled by Dr JA Kirkegaard, Project Leader; 19-21 May Mareeba, 2004)

- ***Bacterial Wilt Studies*** - Several Brassica species along with a commercial product called VOOM[2] were tested in the Philippines and in Australia and the summary findings are attached below in figure and tables (these *tables 1 & 2* and *figure 4* are directly taken from the ACIAR report).

[2] The VOOM contains 20% 2-propenyl ITC (the one present in mustard tissues) in emulsifiable canola oil. The product can be diluted in water and added to the soil as a drench through trickle irrigation to control soil-borne disease. For our purposes, as well as testing a potential new product, the VOOM provided an easy way to determine the concentration of ITC required in soil to suppress BW.

Table 1. *R. solanacearum* populations (cfu) isolated from soil at different times after incorporation of leaf tissues from various Philippine vegetables.

	R.s. population (cfu) per gram soil at different sampling time					
	5% Plant Tissue			10% Plant Tissue		
Treatment	10 days x 10^4 cfu	20 days 10^3 cfu	30 days 10^3 cfu	10 days 10^4 cfu	20 days 10^3 cfu	30 days 10^3 cfu
Control	146.7a	410.7 a	296.7 a	113.3 a	583.7 a	365 a
Pechay (Pavito)	55.3 bc	60.5 f	153.3 c	77.7 b	52.5 f	90 b
Hybrid cabbage (Cabuko)	49.0bcd	36.8 g	230.0 b	2.9 g	29.5g	17.5 d
Sweet potato	4.3 d	95.0 e	29.3 g	18.7 f	63.3 e	17.2 d
Mustaza (Monteverde)	23.0 d	23.9 h	24.7 h	32.3 d	90.8 c	8.0 f
Radish (Speedy)	33.7cd	110.7 d	50.8 d	27.3 e	82.0 d	18.2 d
Chaism (Tosakan)	80.0 b	288.3 b	35.0 e	70.0 c	22.0 h	23.8 c
Cauliflower (Montblanc)	55.0 bc	141.7 c	32.3 f	17.0 e	98.8 b	11.5 e

Table 2. *R. solanacearum* populations at Day 10 and Day 30 following incorporation of various Brassicaceous leaf tissues. The major GSL types and concentration for each tissue are shown

Species	GSL type and concentration (umoles/g tissue)	Day 10 (Log x+1)	Day 30 (Log x+1)
B nigra	2-propenyl (42)	7.2 a	1.6 a
B carinata	2-propenyl (26)	7.2 ab	2.7 abc
B juncea	2-propenyl (14)	7.8 abc	2.6 abc
B carinata	2-propenyl (29)	8.0 abc	2.8 abc
B napus	3-butenyl (0.4)	8.2 abcd	3.1 abc
R sativus	4-methsulbut (16)	8.3 abcde	3.8 cd
Broccoli	Various (2.4)	9.5 cdefg	1.7 ab
Soil only	None	9.3 bcdef	6.3 e
Sorghum	None	9.6 cdefg	6.7 e
Tobacco	None	11.3 g	1.2 a

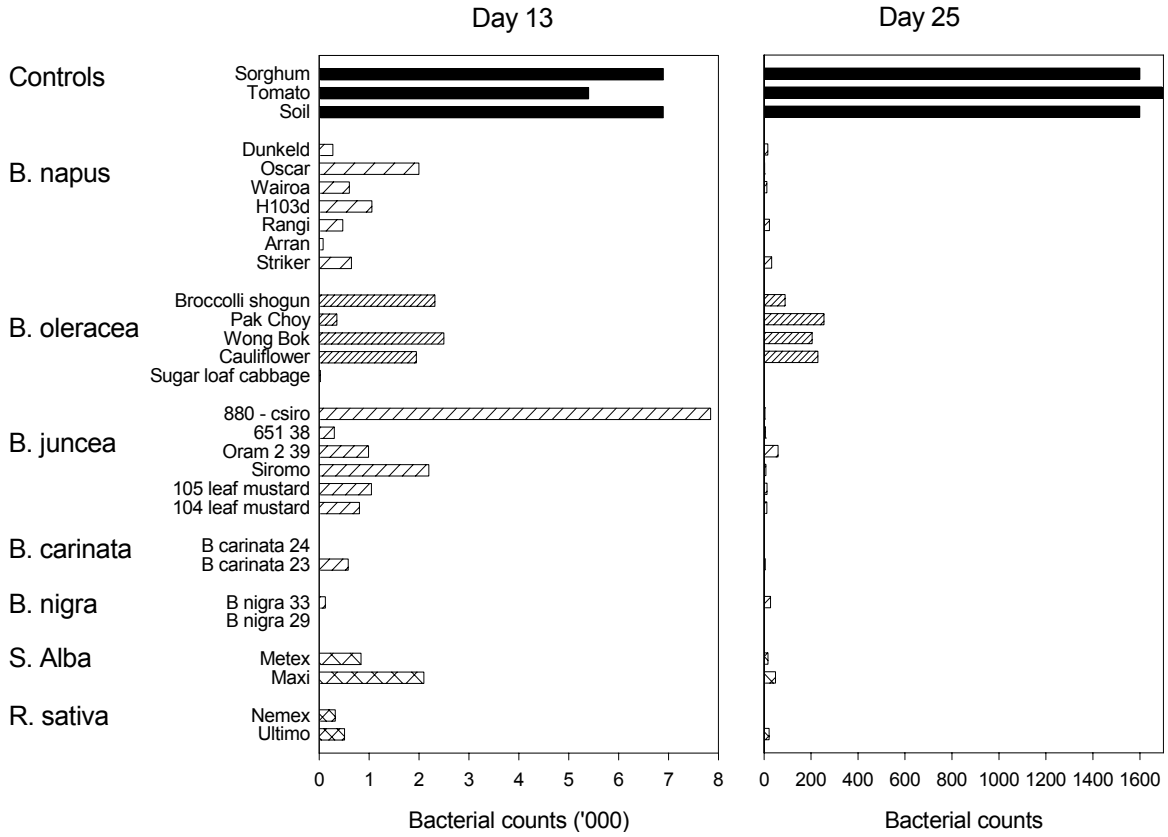


Figure 4 The impact of *Brassica* shoot tissues on populations of *Ralstonia solanacerum* (colony forming units) in sealed containers compared with sorghum, tomato and soil-only controls. The various brassicas are grouped according to species (ie GSL types), and within each species are arranged in ascending order of GSL concentration from top to bottom (ie for *B. napus*, Dunkeld had lowest GSL and Striker highest GSL). This arrangement allows visual assessment of correlations between GSL type, concentration and bacterial suppression.

Box -4: ***Potential useful findings from the AICAR project work on Biofumigation for IPM Trainers***

- Bacterial Wilt was significantly reduced by mustard green manures using best-bet management on a sandy soil, and tomato yields were increased from 4 to 20 t/ha suggesting that further field testing to adapt the concept to specific farming systems is warranted.

- Experiments designed to focus on short-term impacts of volatile ITCs showed that mustards containing higher levels of volatile ITCs were more effective than other species such as rapeseed at suppressing the pest organisms.

- In some cases, macerated *Brassica* tissues amended at realistic field application rates (5% W/W) were as effective as commercial fumigants (e.g. MITC), in reducing both Bacterial Wilt and Root Knot Nematodes to undetectable levels, while non-*Brassica,* or soil-only controls had no impact.

- Longer-term field incubation studies (up to 30 days), where tissues were not macerated (ie minimal ITC release) showed that non-*Brassica* amendments (sweet potato, spinach, tomato, sorghum) were sometimes as suppressive as *Brassica* amendments to Bacterial Wilt and Root Knot Nematodes suggesting that suppression related to the general impact of soil organic matter amendments on soil microbial communities, possibly favoring pest antagonists.

- Laboratory and field experiments confirmed that the suppressive impacts of ITCs were enhanced by ensuring maximum tissue disruption at the cellular level to maximize the reaction between GSLs and the enzyme myrosinase which hydrolyses GSLs to release ITCs.

- In the field, using a mulcher to macerate tissues before incorporation increased ITC release into soil 10-fold compared to rotary hoeing, while combinations of maceration and heavy irrigation could increase ITC levels in soil by 100 fold.

- The suppression based on ITC release from brassicas is enhanced by growing at least 5 kg/m^2 of biomass, providing maximum tissue maceration, rapid incorporation, sealing to avoid volatile loss (with plastic or irrigation) and ensuring adequate soil water is available to facilitate hydrolysis.

- For Bacterial Wilt (BW) management, Mustard (*Brassica juncea*) was the most effective biofumigant followed by white mustard (*Sinapis alba*), radish (*Raphanus sativus*) and rapeseed (*B napus/campestris*), while the levels following a soybean green manure did not differ from the untreated fallow controls in which 100% of plants wilted. In The Philippines Broccoli was also effective in Bacterial Wilt suppression, particularly when using roots and shoots.

- The project conducted an experiment in a tomato crop on a commercial farm (Endeavour Farm) near Mareeba in 2003/4 where BW had been a problem. The heterogeneous distribution of the BW within the paddock (ie hot spots) in relation to the treatments created large variability in the data and no clear evidence of BW suppression emerged.

- Studies on the suppression of BW by VOOM showed that 2-propenyl ITC at 200 - 1000 nmole per g soil was required to suppress BW depending on soil type (higher in soils with high clay and organic matter content due to inactivation of some ITC).

- In a study on biofumigation potential for suppression of the Root Knot Nematode (***M. javanica***), it wa shown that incorporation of Brassica tissue at a rate of 5% (w/w) was much more effective than at 2% in reducing nematode numbers and galling index on tomatoes. Significant differences between the various *Brassica* species emerged after 3-4 weeks.

- Radish is the poorest host of Root Knot Nematode so that populations do not build up during it's growth. Incorporated tissue of radish also suppressed nematode numbers so that radish is an excellent biofumigant crop for nematode control.

6. Success stories related to Biofumigation

The following Biofumigation success stories have been reported:

- ⇒ *Brassica* residues have a suppressing effect on common scab disease (*Streptomyces scabies)* of potato ((Reinette Gouws and Nico Mienie, 2000);
- ⇒ Biofumigation has a similar efficacy as Methyl Bromide in Root-knot nematode *(Meloidogyne* in*cognita)* control in Pepper. (A. Bello, J.A.López-Pérez and M.Arias, 2001);
- ⇒ High concentrations of *B. juncea* can control Masked chaffer beetle larvae (*Cyclocephala sp*) and can be used as a an alternative soil fumigant to methyl bromide (Ryan R.P. Noble and C.E. Sams, 1999);
- ⇒ Highest tomato yields were produced on plots treated with a Biofumigation treatment. (Stephanie G. Harvey and Carl E. Sams, 2001);
- ⇒ Biofumigation can control nematode population (Antonio BELLO et al, 2002);
- ⇒ In the USA, mustard green manure crops have replaced Metham Sodium in potato rotations for disease suppression, with additional improvements in water infiltration, soil organic matter and erosion control (McQuire 2004, 1st International Biofumigation Symposium, Florence, Italy- April 2004)

7. Vegetable Farmer's Knowledge and Research Gap

Below are listed some major research gaps experienced at farmers levels related to Biofumigation:

- ⇒ Farmers do not know which variety they should grow in their region to achieve Biofumigation effect;
- ⇒ What methods of incorporation of Brassica tissue are appropriate;
- ⇒ When they should incorporate (timing);
- ⇒ How frequently do they need to repeat the procedure;
- ⇒ Lack of knowledge on the concentration of glucosinolates in different plant parts (root, stem, leaf, seed, flower etc.).

8. Future Prospects of Biofumigation in Asia

The following features of Asian agriculture highlight current practices and prospects of Biofumigation:

- ⇒ In many parts of Asia, Brassicas are grown intercropped traditionally with wheat and legumes and main crops always perform better in the presence of Brassica. For example, in many parts of India, mustard meal or cake is used to increase the crop health especially in vegetable cultivation such as in eggplant. However, in

most cases farmers have no scientific idea on how the mustard cakes are helping their crops to grow better.

⇒ Brassicaceous crops are widely grown and consumed in all parts of Asia in one way or another. It is grown as an oil seed crop in several South Asian countries whereas in other countries it is grown and consumed as leafy-vegetables, fodder etc. by farmers.

⇒ The content of GSLs varies from variety to variety and differs widely in various plant parts. There are twelve different cultivable species of Brassica available in Asia and they all have cross compatibility, giving chance for varietal improvement for higher glucosinolates concentration.

With the available traditional knowledge of achieving Biofumigation, coupled with availability of a wide range of genetic variation in Brassicaceous crops commonly grown in the region, Biofumigation presents itself as an ecologically safe and economically sound option for soil-borne pest and disease management to vegetable small holders in Asia. Additionally, vegetable cultivation consumes a large share of pesticides compared to many other crops in the region. Farmers do not have any alternative to control soil-borne diseases except using chemicals, such as Methyl Bromide, which is going to be banned by 2005 because of its hazardous effects on the environment. If the present research gaps with Biofumigation could be minimized, it could well evolve as a good option for farmers

To achieve a higher degree of use of Biofumigation at the farmers level, the following key research areas need strengthening:

⇒ Developing agronomic methods of incorporation of Brassica tissue in soil to maximize the Biofumigation effect in different farming systems (plant extract, green manure crop, inter-cropping etc.);
⇒ Identification of appropriate species of Brassica having high amount of GSLs in their tissue;
⇒ Selecting/breeding superior cultivars having high GSLs for the purpose of Biofumigation;
⇒ Utilization of the bio-products of the mustard oil industry such as mustard meal, powder etc.;
⇒ Extension and refinement of the traditional knowledge available at farmers domain on the use of Brassica intercropped with other vegetable crop;
⇒ Long-term research to learn the susceptibility of different soil microbes to the Biofumigation.

9. Experience from IPM Training Settings

9.1 Cambodian experiences with Biofumigation

The Cambodian trainers took the lead in developing the first-ever exercises and training guides on the management of soil borne diseases at the 2003 Siem Reap TOT. They developed a range of small exercises and pot experiments to learn and familiarize themselves with the concept and ideas related to bio-fumigation. The detailed trials and other small studies are attached in *annex-1*. Apart form using Biofumigation on diseases, the trainers and participants at Siem Reap TOT, explored the potential of a mixture of Brassica leaves extract, alcohol and water for management of insect-pests. The different Brassica species, alcohol (local rice alcohol) and water were grounded together followed by spray of solution on the leaves before feeding it to common insect-pests of Brassica crop. Results form the experiments are summarized in *Box-5*. Water and alcohol were used as control in this experiment.

Box 5: Result of Use of Brassica extract against Diamond Back Moth (DBM) of Crucifers at Training of Trainers Course, Siem Reap, Cambodia (November 2002 – April 2003)

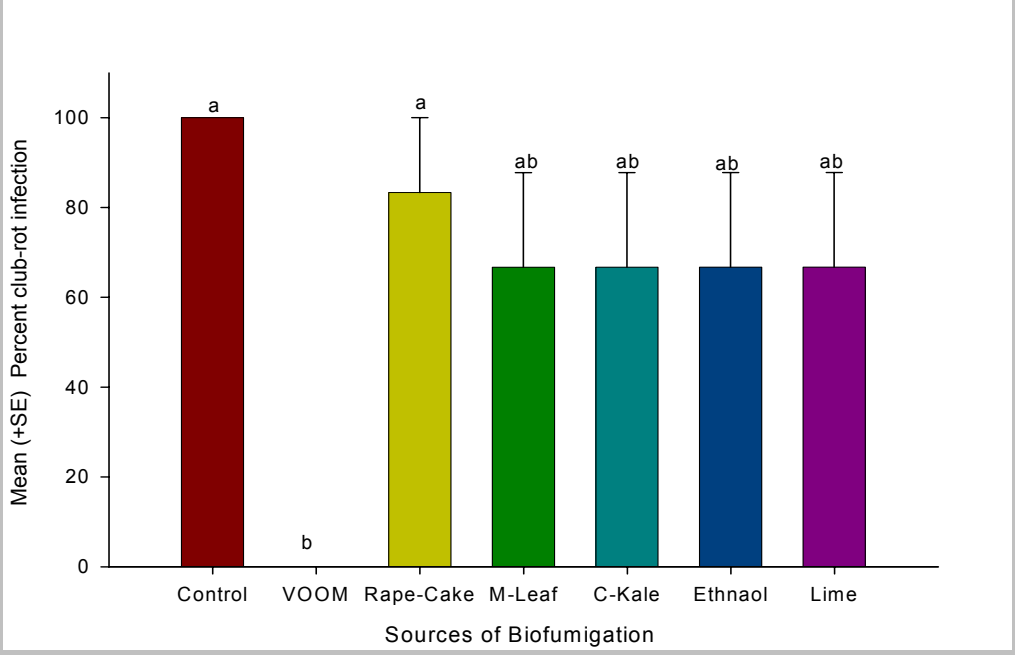
Source Brassica species	*Pure dilution (+ larvae) (Treatment)*	*Alcohol (+ larvae) (Control)*	*Water (+ larvae) (Control)*
Cabbage	DBM larvae did not die	DBM larvae did not die	DBM larvae did not die
Chinese Kale	9/10 larvae died 1/10 larvae pupated	DBM larvae did not die	DBM larvae did not die
Green Mustard	3 out of 5 DBM larvae died All (5/5) Armyworm larvae alive	DBM larvae did not die	DBM larvae did not die
Pak-choi	DBM larvae did not die	DBM larvae did not die	DBM larvae did not die

Conclusion by the Cambodian TOT participants: Cabbage and Pak-choi extracts are not effective against DBM while green mustard showed a little effect on DBM but not in armyworm, while Chinese kale extract was proven most effective against DBM. The experiment should be repeated to prove the efficacy of the extracts.
(Source: delacruz, Luci: A report of the Training of Trainers Course, Siem Reap, Cambodia & Personnel communication. Nov. 2002)

9.2 Chinese Biofumigation experience

The concept of Biofumigation was first introduced in the IPM Training of Master Trainers (TMOT) in Yunan followed by a Training of Trainers (TOT) held earlier in 2004 in Yunnan province. Since China posses varied types of Brassica crops and crop by products, new source of Biofumigation like Mustard oil cakes were tested along with Brassica crops and VOOM (20% ITC; Volatile Oil Of Mustard-VOOM), a commercial Biofumigation product from Australia. The details of experiments design conducted are attached in *annex 2 & 3*. In a pot study with Chinese cabbage, when soil was pre-treated with various sources of Biofumigation (locally available) along with VOOM; VOOM treated pots resulted into 0% infection compared to other treatments. No other treatment including lime gave satisfactory control. The detail results are included in the *Box 6*.

Box 6: Effect of various Brassica sources on the % club-root infestation (Plasmodiophora brassicae), Chinese Cabbage crop, 2004 spring season, Xundian, Yunnan, P.R. China



Fig; Mean (±SE) percent club-root infection caused by pre-planting soil treatments with different sources of Biofumigation); column marked with the same letter are not statistically different (F = 3.29; df= 6,35, P = 0.013; Tukey's HSD test)

Participants in the Kunming TOT, also tested the effect of various Biofumigation source along with VOOM on the incidence of Bacterial soft rot (*Erwinia* sp) and detailed results are included in *Box 7*. No incidence of the bacterial soft rot was recorded in the VOOM treated pots.

Box 7: Effect of various Brassica source on the % infected plant with soft rot (Erwinia sp.) on Chinese cabbage, Training of Trainers Course, 2004 spring season, Xundian, Yunnan, P.R. China

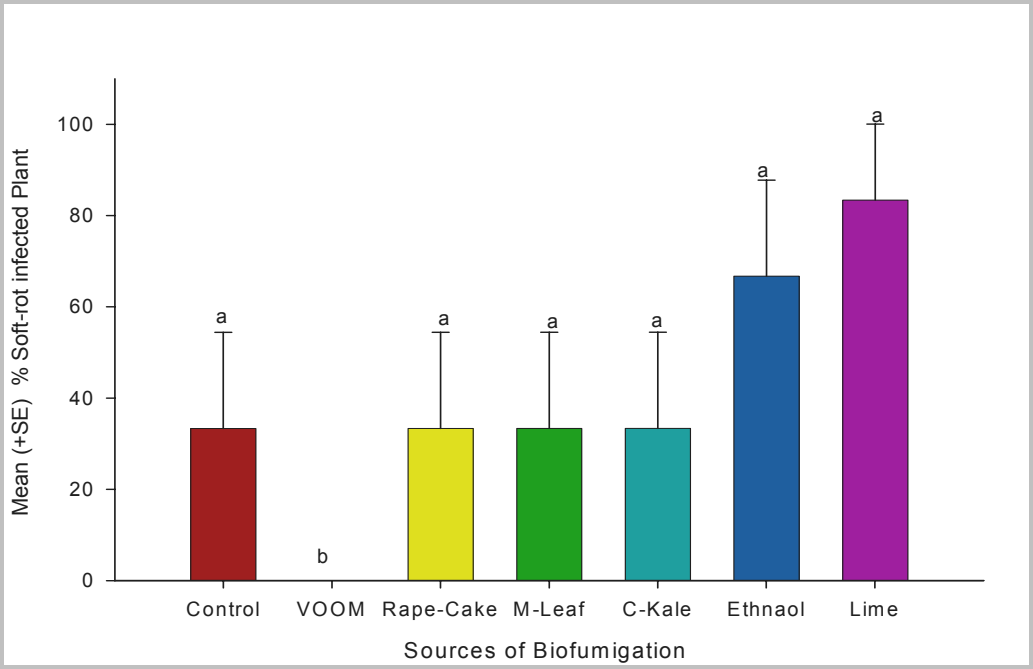


Fig. Mean (±SE) percent club-root infection caused by pre-planting soil treatments with different sources of Biofumigation); column marked with the same letter are not statistically different.

The length of the tap root was measured for all treatments as an indicator for root health. Obvious differences were found in the root system under the various treatments, both with regard to the length of the tap root and the development of the fibrous roots. Tap root length was significantly longer in the VOOM treatment compared to all other treatments (*see figure 5 & 6*). The details could be seen in *Box 8* below.

In summary, the ITC extract VOOM was the only treatment that was able to effectively kill the club root and soft rot inoculums in the soil. None of the biological sources of ITC had enough of it released to manifest the same results. This can be due to either too low levels of ITC in the various materials, or to inadequate preparation of the materials not allowing it to release the full ITC content to the soil to effectively attack the pathogens.

Box 8: *Effect of various Brassica source on the length of tap-root system (cm), Chinese cabbage, Training of Trainers Course,* 2004 spring season, Xundian, Yunnan, P.R. China

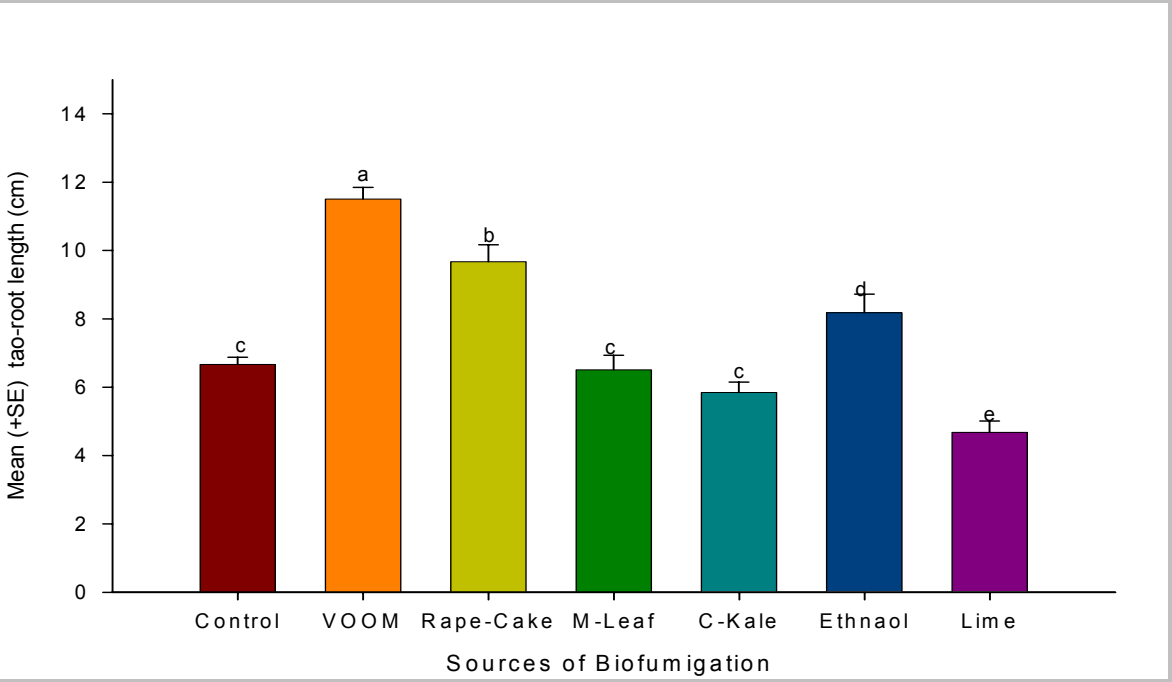


Fig.: Mean (±SE) tap-root length (cm) caused by pre-planting soil treatments with different sources of Biofumigation); column marked with the same letter are not statistically different (F = 36; df = 6, 35; P<0.001; DMRT test).

Figure 5. Isolated roots of Chinese cabbage plants in Biofumigation trial (After Elske, 2004)

Figure 6. Plants at 39 days after sowing. Sequence of treatments: control, Voom, rape cake, Chinese kale, lime, mustard, and alcohol. (After Elske, 2004).

10 References

A.Bello, J.A.López-Pérez and M.Arias.(2001).*Biofumigation and grafting in pepper as alternative to methyl bromide.* Centro Ciencias edioambientales,

Brown P.D. and Morra M.J. (1997). *Control of soil-borne plant pests using glucosinolate containing plants.* Advances in Agronomy 61, 167-231.

Elske van de Fliert . (2004). *Effect of Biofumigation on clubroot and soft rot in Chinese cabbage 2004 spring season, Xundian, Yunnan,* P.R. China. FAO Regional Vegetable IPM Program, Phase – II, Vietnam

Kirkegaard J.A. (2004). *ACIAR Review Report LWR2/2000/114, Evaluating Biofumigaiton for Soil-Borne Diseases Management in Tropical Vegetable Production.*

Kirkegaard, J. and Matthiessen, J., 2004-5. Developing and refining the Biofumigaiton concept. Agroindustria / Vol. 3 / Num. 3 2004 5.

Matthiessen J.N. and Kirkegaard J.A. (1998). *Biofumigation for managing soil-borne pests - progress, pitfalls and prospects.* In: Proceedings of the 6th Australasian Applied Entomology Research Conference (Eds M. Zalucki, R. Drew and G. White Vol. 1 pp 364 - 372 (University of Queensland).

Reinette Gouws and Nico Mienie (2000**)** *Biofumigation of Common Scab of Potatoes in the Republic of South Africa.* Agricultural Research Council-Roodeplaat, Private Bag x293,Pretoria, 0001, South Africa.

Ryan R.P. Noble and C.E. Sams (1999) *Biofumigation as an alternative to methyl bromide for control of white grub larvae.* Department of Plant and Soil Science, University of Tennessee, Knoxville, TN 37901

Stephanie G. Harvey and Carl E. Sams (2001). *Brassica biofumigation increases marketable tomato yield.* Department of Plant Sciences and Landscape Systems, University of Tennessee, Knoxville, TN 37901

11 Some suggested websites to obtain more information on Biofumigation:

1. www.ento.csiro.au/research/pestmgmt/ soil_pests/**biofumigation**_info.html
2. www. mbao.org/2001proc/031%20Bello%20A%20San%20Diego-Sept%202001.pdf
3. www. mbao.org/2003/006%20DaugovishOmbao-charts.pdf
4. www.faculty.ucr.edu/~atploeg/ Ploegweblinks/Solarization.html
5. groups.ucanr.org/.../Biofumigation,_ Soil_Microbial_Communities,_and_Plant_Nutrient_Up.htm
6. www.regional.org.au/au/asa/1998/5/267kirkegaard.htm - 18k
7. www.sardi.sa.gov.au/pages/horticulture/

   pathology/hort_pn_biosoilstatus.htm:sectID=336&tempID=111
8. groups.ucanr.org/.../Biofumigation,_ Soil_Microbial_Communities,_and_Plant_Nutrient_Up.htm

ANNEXES

Annex 1: Session guide on Biofumigation in Cambodia

Background:

All *Brassica* species contain Glucosinolates (GSLs) in varied proportion. The content of GSLs varies from species to species and from one plant part to the other plant part. Indian mustard (*B. juncea*) contains significantly higher proportion of GSL compared to other *Brassica* species. Pioneer work done by scientists of CSIRO in Australia revealed that using such plant species could significantly reduce soil borne diseases. The use could vary from using such crops as a green manure crop to direct incorporation of macerated plant tissues.

When this secondary sugar is exposed to the enzymes and water, it turns in to the ITC (Isothiocyanates). ITCs could in turn fumigate the soil and act as biocide. The phenomenon is termed as "Biofumigation" by Australian scientists.

Materials required:

Leaf materials of different species of Brassica species like Cabbage, Chinese kale, Mustard, Grinder or mixer, Alcohol (may or may not be needed depends on availability) and known bacterial and/or fungal infected patch of land, Seedlings of tomato or other vegetable species. (For the TOT, the participants can use old seedbed, which are infested with Bacterial wilt and damping off).

Time Required:

One week for the incorporation of the Brassica plant materials. Another 12 weeks to learn the effect (in the case of tomato bacterial wilt trial). While in case of Seedbed damping off trial 4-6 weeks would be needed.

Procedure:

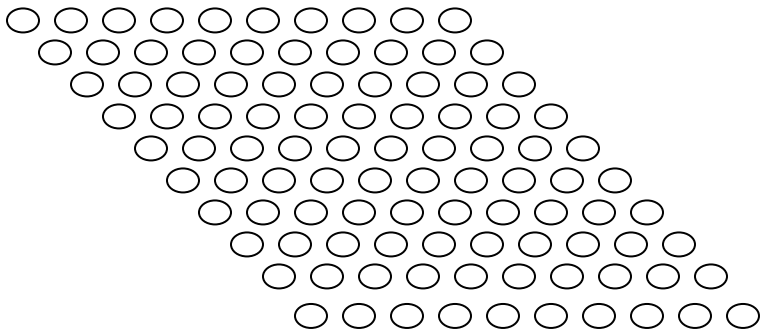
A. Procedure for setting of trials on the bacterial wilt management in tomato crop.

1. Sufficient quantity of the cabbage leaves will be collected from the existing field.
2. The leaves will be chopped and will be grinded with 20% available local rice whisky (minimum 40% alcohol).
3. Immediately after, the green slurry will be incorporated in the soil and covered with the soil.
4. Sufficient moisture will be ensured for the remaining period until transplanting.
5. Two weeks after incorporation of cabbage leaves slurry, the transplanting will be done. (Some groups are planning to transplant immediately after the incorporation of masticated cabbage leaves).
6. Each group will get three small plots (each plot will accommodate 32 plants each). Each group will have two treated and one control plot for the study.
7. All groups will use KK_1 variety, which is highly susceptible to Bacterial Wilt.

8. After, transplanting normal after-care operations will be performed.
9. Weekly plant mortality should be counted and recorded.
10. Cause of death of plants should be verified with the simple bacterial wilt "ooze" test.

B. Procedure for the damping off trial in seedbed

1. Similar to the above-mentioned trial, cabbage leaves will be chopped, grinded and mix with sufficient quantity of alcohol to turn into green slurry.
2. This green slurry will be immediately incorporated in to the seedbed having a history of damping off.
3. After incorporation, sufficient moisture regime should be ensured until seeding.
4. Each group should cultivate two plots of 1-meter square size. One such plot will be treated and the other is to be used as control.
5. One week after treatment, in treated plot, one hundred counted number of tomato seeds will be seeded in following manner:



6. Number of seeds germinated will be counted to determine the pre-emergence mortality. The Pre-emergence mortality could be calculated by using following simple calculation:

   Pre-emergence mortality %
   = Total number of seeds not germinated / Total seeds used X 100

7. Similarly, post–emergence mortality could be determined by counting total number of surviving seedling at the end of week –4 (25-30 days old).
   Post-emergence survival percentage
   = Total number of healthy seedlings at week 4 / Total number of seeds germinated X 100

(The number could be counted on weekly basis and the result could be shown by a simple graph, having (weeks) on x- axis and surviving plants or dead plants in y-axis).

Endnote:

1. These trials are being implemented for the first time in any of such training setting and methods have not been perfected. Thus it is strongly suggested that:
    a. Sufficient amounts of the *Brassica* materials should be incorporated;
    b. Incorporation should be done immediately (owing to volatile nature of the ITC, any delay would reduce the ITC content in the slurry) after grinding of the leaves and green slurry should be properly incorporated inside the layers of soil;
    c. Sufficient moisture should be ensured in treated plot so that Glucosinolates can be easily converted in to the ITCs (facilitation of hydrolysis);
    d. It should be made abundantly clear to the participants that they need to further perfect the amount of materials, Brassica species etc to maximize the effect. Further reading materials are to be made available on the subject;
    e. Seeds used for the Damping off trial should have 100% germination capacity. The seeds that are to be used in the experiment should have a known germination capacity. It should come from a known source with a known level of germination. In the case local seeds are to be used, it is highly suggested to do a simple germination test (*see CABI/FAO Training Manual-2A-10*) and then adjust test results accordingly.

Annex 2: Brassica extract against Brassicaceous Insect-pest

Background

Plant species are known to produce groups of secondary plant chemicals to ward off insect-pests and other biotic stresses. Most insect-pests developed genetic ability through the course of evolution that equipped them with genes to counter such chemicals to survive and thrive. All *Brassica sp*. are producing Glucosinolates that could potentially be lethal to the DBM and other insects that feed on it. However, these insects have a gene that breaks the Glucosinolates before it can convert into ITCs and thus remain unharmed. Nevertheless, if, these Glucosinolates could be turned in to ITCs and sprayed on plant directly, DBM would not be able to remain unharmed.

Objective:

To learn the biocidal ability of the Brassica extract against DBM and *Heliothis* sp. larvae.

Time required:

For small insect-zoo studies – 5-6 days

Materials required:

Leaves of Cabbage, Chinese Cabbage, Green Mustard, Green Petiole and Chinese kale @ 1 kg each, 20% alcohol@ 200 ml per group per kg of Brassica species for better extraction of Glucosinolates, Strainer / Cotton cloth, Sprayer, water, DMB larvae (50 per group), *Heliothis* larvae (50 per group).

Procedure:

1. Each group will select one Brassica sp. and take a Kg of plant material each.
2. 200 ml alcohol (20%) and 50 ml water.
3. The leaves with water and alcohol should be properly grinded into very fine textured slurry.
4. The slurry so obtained should be strained though either a strainer or piece of cotton cloth to get clear liquid.
5. This undiluted clear liquid then should be sprayed on the potted cabbage plants and sufficient time should be allowed until the spray is dried up on leaves.
6. After that, 10 larvae of DBM or *Halitosis* should be released to feed.
7. The feeding and other general behavior of the larvae will be noted daily for a period of 3 days. Then results should be processed for example making use of below mentioned leading questions.

Leading questions:

1. Have you observed any change in feeding and general behavior of larvae after first day of release? If so then why?
2. How many larvae died after first day of release and why?
3. How can you potentially use this experience for DBM mgt. in Cabbage field in FFS and Farmer's club in your area?
4. What other common sources of *Brassica* species are available for this purpose?
5. What practical problems could farmers potentially face in using this method for DBM and other pest mgt?
6. What other ideas do you have to improve the killing efficiency of the extract?
7. What would be the effect of such spray on the population of natural enemies in the field?
8. Does any thing happen to the leaves after the spray? Was there any evidence of phytotoxic effect noticed? If there were such an effects, what you would do to minimize such effect?

Annex 3: Session Guide on Biofumigation, China

Background:

Brassicaceous crops and other Brassica by products like oil meal cakes are known to suppress the club root disease. Similar results were obtained by using VOOM (a commercial preparation containing 20% ITCs) against Club root (*Plasmodiophora bassicae)* in New Zeeland. Participants of the Training of Trainers will explore the potential of Brassica plant parts and other locally available by products like oil meal cake along with VOOM against club root disease.

Time required:

5-6 weeks

Materials needed:

Leaves of the all-available crops (*Mentioned below as treatments*.) @1 kg/5 kg soil, 50 ml alcohol, plastic wrapper, mustard meal, pots, infected soil from the TMOT plots were disease

Planned treatments:

Following Brassica crops (treatments)

1. Broccoli leaves
2. Chinese Cabbage
3. Cauliflower
4. Mustard leaves
5. Mustard Cake
6. VOOM (1: 1500-2000)
7. Control (a non Brassica crop)

General procedure for all experiments:

1. Clean pots with 5 kg infected soil from the TMOT plot, where a desired disease is present;
2. Chopped (macerate tissues) with alcohol and water (If 6 different Brassica are used then each will be a treatment and VOOM (1:2000) will be 7^{TH} treatment.);
3. Mix with soil in pot (Soil *should be collected from the infested patch in the field);*
4. Put water to wet the pot;
5. Cover the top of pot with plastic membrane;
6. Leave for 10 days (not outside as temperature is low. Preferably around house);
7. Transplant seedling @ four per pot. Regularly provide water and nutrient to the pots to keep the potted plants growing.

Observation/data collection:

a. For club root - After 4-5 weeks uproot the plants and compare the root system and clubs in the root of the cabbage (percentage infested plant);
b. For bacterial wilt – keep counting the dead plants/week and confirm the cause of death of each plant by ooze test;
c. For bacterial soft rot as well, count weekly-infested plant/dead plants.

Analysis:

Prepare a simple line graph of mortality percentage and/or infected %/week to deduce the outcomes and process the results by following leafing question. It is highly suggested that use mean± SE (Std. error of mean[3]) while calculating the mortality and infection percentages as it would provide you more in-depth idea about the trends.

Leading Questions:

- Was there any difference in the root system of the treated plants with control? (In all three experiments)
- Why you think treated plants developed a better root system than control?
- Is there any difference in the diff. Brassica species and VOOM?
- How you could maximize the retention of GLS in the soil to maximize benefits?
- Which species of Brassica performed better than others and why?
- What other method of incorporation and Brassica species would you like to test and why?
- What other major soil borne problems are you expecting to encounter in your working area and do you have any idea of small experiments with the farmers related to bio-fumigation?
- Do you have any other source of Bio-fumigation available?

[3] Std. error of mean could be simply calculated by Std. deviation/ Sq. root (n); where n = number of observation. MS Excel can easily calculate mean and std. error of mean.

Annex 4: Pot studies on Management of the club-root disease, Kunming, Yunan Province

Background:

Preliminary studies with VOOM (a commercial preparation containing ITCs) against Club root in New Zeeland showed promising results. Biofumigation of the substrate by using locally cultivated mustard species and/or by using products from the mustard oil, industry (like oil cake) could also potentially provide good management option against club root disease.

Time required:

10 days for incorporation and 30 days (4 weeks for data)

Materials needed:

Plastic pots (1 kg.) size 60 numbers, infected soil from the club root fields, alcohol, water, seed of the cabbage (with proven good germination), lime, VOOM (20% P-ITC), a blender.

Planned treatments:

1. Leaf Mustard (250 g plant part + 50 ml alcohol +250 ml water);
2. Mustard tuber (250 g plant part + 50 ml alcohol +250 ml water);
3. Mustard oil cake (50 gm + 50 ml alcohol +250 ml water;
4. VOOM (1: 1500-2000) – 5-10 µ l /1 kg soil;
5. Control (a non Brassicaceous crop) ;
6. Lime (dose rate 100 kg/mu).

General procedure steps for experiments:

- ⇒ Fill pots with 1 kg infected soil from the infected field (if not available, finely chop the clubs from the infected soil and mixed well in pots and keep it for few days (10 days) with moisture and cover it with plastic sheet)
- ⇒ Every group should set up their own sets of treatments, which would lead to 6 replication of the experiment.
- ⇒ Chop plant parts (macerate tissues) with alcohol and water and make slurry.
- ⇒ Mix the slurry thoroughly with the soil in pot and mark the pots for treatments and dates and time.
- ⇒ Apply water, if needed and cover the pots with plastic membrane for 10 days.
- ⇒ Put 10 seedlings of the a desired variety (cabbage) in each pot
- ⇒ After 30-40 days, irrigate and loosen the soil with water and then gently uproot the plants, wash the root zones gently and observe for the symptoms of the club formation.

Observation/data collection:

After 30-40 d of seeding, observe number of plants/pot that has developed clubs.

Data Table:

Treatment	Replication	Club root	No club root	% club root
Control	R1			Club root/10 X 100

⇒ Calculate Average % club root per treatment and also calculate standard error of mean (SEM) = standard deviation/square root (n)
⇒ Plot a graph with mean % club formed plant for all treatment with SEM. Draw conclusions

(For more enthusiasts, run a one way ANOVA followed by a post-hoc by Turkey's HSD and then draw conclusions).

Leading Questions:

⇒ Was there any difference in the root system of the treated plants with control? If yes, why? What in your idea was the reason?
⇒ Why you think treated plants developed a better root system than control?
⇒ Was there any difference in rate of club formation in diff. Brassica species and VOOM treated pots and why?
⇒ How you could maximize the retention of GLS in the soil to maximize benefits?
⇒ Which species of Brassica performed better then others and why?
⇒ What other method of incorporation and Brassica species would you like to test and why?
⇒ What other major soil borne problems you are expecting to encounter in your working area and do you have any idea of small experiments with the farmers related to bio-fumigation?
⇒ Do you have any other source of Bio-fumigation available?

Annex 5: Damage symptoms of some common soil borne diseases

Fig 1: Club root symptoms on field cabbage, March 2004, China PR

Fig 2: Club root symptoms

Fig 3: *Sclerotia* blight of yard long bean, Cambodia

Fig 4: Stem rot of Mung Bean, Cambodia

Fig 5: Southern blight of French bean, Vietnam

Fig 6: Southern blight of French bean, Vietnam

Fig 7: Bacterial Wilt of Tomato, Lao PDR

Fig 8: Bacterial Wilt studies by the farmer's group (variety trial), Lao PDR