# INFLUENCE OF INTERCROPPING SYSTEMS ON SOME MEDICINAL PLANTS IN THE PRESENCE OF BIOFERTILIZATION

**Nour El-Dein, M.[1] and S.I. Younis[2]**
**[1] Soil, Water and Environmental Research Institute, A.R.C., Giza**
**[2] Medicinal and Aromatic Plants Lab., Dokki Hort. Research Institute, Giza.**

## ABSTRACT

Two field experiments were conducted at El-Nubaria region, El-Hussein village, seasons 2002 and 2003 to investigate the influence of intercropping of mustard and/or nostortium plants on fenugreek plant species in the presence of biofertilization, on seeds yield and some chemical components of these plants.

Some intercropping systems were carried out. All treatments were inoculated with mixed inoculum composed of some $N_2$-fixing and phosphate-dissolving microorganisms as well as fertilized with poultry manure except traditional treatment as control. The increase in fenugreek seeds yield as caused by the intercropping treatments were as follow : $T_7$ (fenugreek in side of ridge + nostortium, in other side + mustard, in ridge farrow) and $T_3$ (fenugreek, in both side of ridge + mustard in ridge farrows). The above mentioned treatments ($T_7$ and $T_3$) had also the best effects on seeds yield of mustard and nostortium compared with other intercropping treatments. On the other hand, $T_7$ and $T_3$ treatments gave lower seeds yield as compared to solid treatments (fenugreek, mustard or nostortium pure stands) which could be atributed to the more density of plants for solid planting as by intercropping one. Intercropping treatments increased land equivalent ratio (LER) and $T_7$ and $T_3$ gave the highest values (1.92 and 1.73) in the first season and (1.97 and 1.34) in the second season. Weight of 100 seeds did not varied. Intercropping increased seeds contents of N and P especially $T_7$ and $T_3$. Biofertilization contributes also in increasing seeds yield and their content of N and P. This indicates that biofertilization of intercropped plants improved their growth and may be helpful in increasing efficiency and succession of intercropping process.

**Key words**: intercropping, biological fertilization, medicinal plants.

## INTRODUCTION

Medicinal plants is important in therapy as herbs or ingredients. They contain different types of active materials. Mustard, for example, contains sinigrin "glycosilate" and myrosin enzyme. Seeds, also, contain fixed oil, protein, micilage and traces of sinepine hydrogen sulphate. It used externally as rubefacients and counterirritants, and internally as condiment and in full doses as an emetic. Fenugreek contains steroidal sapogenins, fixed oil, mucilage protein, volatile oils and trigonelline alkaloid, and used in nutrition, spicy, lactogogue and for the treatment of diabetes (Wallis, 1985 and Trease and Evans, 1994). Nostortium have mucilage, glucosinolate, carotines, fixed oil and alkaloids (Azzam, 1980). It used in relief pain e.g. tooth, joints, urinary bladder and sciatica, and used also in treatment of kidney, liver, spleen, diarrhea and diabetes (Abdel-Hady, 1998).

Biological fertilization is of interest in agriculture process of medicinal plants to decrease, as possible as, mineral fertilizers allocated in plants tissues and improves crop production (Bhatia *et al.*, 1998 and Kim *et al.*, 1998). $N_2$-fixing and phosphate-dissolving microorganisms had been used in inoculation of different plant types. They save some elements available for plants, thus may contribute in decrease of mineral fertilizers (N and P) used (Sprenat, 1990 and Rodales *et al.*, 1999). Biological plant products is more safe than chemicals treated ones.

Intercropping of non-legume crops on legumes help in decreasing N chemical fertilizers used for non-legumes production. This fact was assessed by Abu-Taleb *et al.* (1999). They reported that legume crops transfer $N_2$-fixed to non-legume ones through direct decompositon of both nodules and fine roots.

Therefore, the current work was directed to study effects of intercropping of non-legume medicinal plants (nostortium and mustard) with legume one (fenugreek) on quality and quantity of seeds yield of these plants in the presence of biofertilization with some $N_2$-fixing and phosphate-dissolving microorganisms.

## MATERIALS AND METHODS

Two field experiments were conducted at El-Hussein village, El-Nubaria, El-Behera Governorate during winter seasons of 2002 and 2003. Seeds of studied plants species; mustard (*Brassica alba,* L.), nostortium (*Lapidium sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.) were kindly provided from Horticulture Research Institute, A.R.C., Giza

Soil of the experiment is sand soil, poor in organic matter and nutrients. It is contents illustrated in Table (1). Poultry manure was brought about from commercial poultry farms and the chemical analysis showed in Table (1).

Cultures of *Azotobacter* spp. and *Bacillus megatherium* were used for inoculation of non-legume plants: mustard and nostortium. While, legume one (fenugreek) inoculated with a mixture of *Azotobacter* spp., *B. megatherium* and *Rhizobium meliloti.*

*Azotobacter* spp. was isolates in laboratory from rhizosphere soil of maize plants using Vancura and Mucura medium (1960) which composed of: sucrose, 30 g; $K_2HPO_4$, 0.16 g; NaCl, 0.2 g; $MgSO_4$-$7H_2O$, 0.2 g; $CaCO_3$, 2.0 g; $FeSO_4$, 0.005 g; $Na_2MO_4$, 0.005; $NaBO_4$, 0.005; distilled water, 1 liter.

*B. megatherium* isolate was obtained from Department of Agricultural Microbiology, Agricultural Research Station, Giza, Egypt, and cultured on nutrient broth medium formed of beef extract, 1.0 g; yeast extract, 2.0 g; peptone; 5.0 g; sodium chloride, 5.0 g.

*R. meliloti* isolate was provided from Bacteriology Lab., Sakha Agricultural Research Station, Sakha, Kafr El-Sheikh and cultured using yeast mannitol medium (Somasegaran and Hoben, 1985). The components of medium were: Mannitol, 10.0 g; $K_2HPO_4$, 0.5 g; $MgSO_4$-$7H_2O$, 0.2 g; NaCl, 0.1 g; yeast extract, 0.5 g; distilled water, 1.0 L.

Inoculation process was performed by mixing the (fenugreek) seeds with a mixture of *B. megatherium* and *R. meliloti* as peat-based (contain $3 \times 10^8$ and $5 \times 10^8$ cells/g, respctively), and *Azotobacter* spp. as liquid culture contains $1 \times 10^8$ cells/ml (100 ml culture/fed.) using appropriate sticking material as Arabic gum period to sowing, then followed by irrigation. But mutard and nostortium seeds inoculated with *B. megatherium* and *Azotobacter* spp. only.

**Table (1):** Mechanical and chemical analysis of the studied filed soil .

| Mechanical analysis | | | Texture class | pH 1: 2.5 | E.C. $(dSm)^{-1}$ | O.M. (%) | $CaCl_3$ (%) | Soluble cations (meq./L) | | | | Soluble anions (meq./L) | | | | Macro elements (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sand % | Silt % | Clay % | | | | | | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{--}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{--}$ | N | P | K |
| 95.55 | 4.45 | - | Sand | 8.3 | 0.22 | 0.29 | 4.6 | 0.8 | -- | 0.8 | 0.33 | -- | 1.2 | 0.1 | 0.63 | 6 | 14 | 140 |

Chemical analysis of poultry manure.

| pH | Soluble salt % | Organic matter % | Total (ppm) | | |
|---|---|---|---|---|---|
| | | | N | P | K |
| 7.90 | 1.11 | 67.5 | 21800 | 13100 | 17000 |

The experiment was carried out in a complete randomize design with four replicates for each treatment (T):

($T_1$): Un-inoculated solid fenugreek or mustard or nostortium (taditional).

($T_2$): Inoculated solid fenugreek or mustard or nostortium.

($T_3$): Inoculated fenugreek, all the ridges + mustard in ridge farrow.

($T_4$): Inoculated fenugreek, two ridges + mustard, one ridge.

($T_5$): Inoculated fenugreek, two ridges + nostortium, one ridge.

($T_6$): Inoculated fenugreek, side of ridge + mustard, side of ridge.

($T_7$): Inoculated fenugreek, side of ridge + nostortium, side of ridge + mustard in ridge farrow.

Poultry manure was added, with the rate of 10 ton/fed., to all treatments except for traditional treatments (un-inoculated solid). It mixed with the surface layer (0.0-20 cm) just before planting. Experimental plots were of distances 4 x 4 with six ridges in each plot. The two ridges in borders were excluded at collecting samples. Solid treatments of each plant species were planted as follow: mustard and nostortium seeds were sown by a rate of 20.0 and 10.0 kg/fed. respectively, in hills in one side of each ridge. Distances between hills were 25 cm for mustard and 20 cm for nostrotium. While fenugreek seeds were sown by a rate of 20 kg/fed. by spreeding on both sides of ridges. But in case of intercropped treatment, the rates of seeds were calculated according to treatment, the rates of seeds were calculated according to planted area from each crop. The sowing patterns were as follow: $T_3$; fenugreek seeds were sown as followed by solid treatments, but mustard seeds were sown in ridge farrow with distances about one meter between hills. $T_4$; two ridges of fenugfollowed by one ridge of mustard and each plant species sowed by the same method of solid ones. $T_5$; two ridges of fenugreek followed by one ridge of no, they planted as followed in solid ones. $T_6$; one side of ridge was sown with fenugreek and the other with mustard. Planting was carried out by the same method applied in solid treatments. $T_7$; one side of ridge was sown with fenugreek and the other with nostortium. Mustard was planted in ridge farrow with distances of one meter hills.

Potassium was added to all experimental units as potassium sulphate with the rate of 15 kg $K_2O$/fed. Phosphorus was added before tillage as calcium super phosphate with the rate of 30 kg $P_2O_5$/fed. for un-inoculated treatment and 15 kg $P_2O_5$/fed. for inoculated treatments. Nitrogenous fertilizers was applied as ammonium nitrate as follow:

a. Un-inoculated solid treatments of legume and non-legume plants dressed with 80 kg N/fed.
b. Inoculated solid treatments of non-legume plants dressed with 40 kg N/fed.
c. Inoculated intercropped and inoculated solid legumes dressed with 20 kg N/fed.

Seeds yield, (kg/fed.) and seed index (g) were determined at harvest time of each crop. Seeds were taken randomly from each treatment and ground to fine powder and were packed in paper bags prior to chemical analysis. Nitrogen was estimated in seeds by micro-Kjeldahl method reported by (A.O.A.C., 1975). Phosphorus was measured colouremetricaly according to (Olsen *et al.*, 1954). All experimental data were statistically analyzed by usual technique of analysis of variance (ANOVA) as mentioned by Steel and Torrie (1980) and was carried out using IRRISTAT software version 3/93. Duncan's (1955) multiple range test was used to compare means at 0.05 level of probability.

## RESULTS AND DISCUSSION

Inoculated mustard plants gave higher seeds yield (483.3 and 500.7) than un-inoculated treatments (452.5 and 453.3) in both seasons, but the differences were not significant (Table 2). All intercropped treatments exhibited lower seeds yield than inoculated and un-inoculated solid controls. Seed index, generally, did not exhibit obvious variations between treatments except for $T_4$ in the first season which gave the highest value (0.84 g). The studied treatments gave large variations in N-content of seeds (Table 2). The highest value (14.9 kg/fed.) was recorded by inoculated solid treatment. P-content showed inconsistent differences between traditional and inoculated treatments. But significant negative variations was noticed between intercropped treatments and traditional or inoculated solid treatments. $T_7$ recorded the highest percentage over intercropping treatments (1.08 and 0.99) in first and second seasons respectively.

**Table (2): Effect of intercropping mustrad plants with fenugreek plants on mustard seeds yield (kg/fed.), seed index (g), N-and P-contents (kg/fed.), under sercunestances of biofertilization.**

| Treatment | Seeds yield (kg/fed.) | | Seed index (g) | | N-content (kg/fed.) | | P-content (kg/fed.) | |
|---|---|---|---|---|---|---|---|---|
| | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 |
| $T_1$ | 452.5 a | 453.3a | 0.67b | 0.69a | 13.4b | 12.3b | 3.42a | 3.40a |
| $T_2$ | 483.2a | 500.7a | 0.75b | 0.68a | 14.9a | 14.1a | 2.59a | 2.3b |
| $T_3$ | 163.2b | 147.3b | 0.71b | 0.69a | 5.4c | 5.4c | 1.02b | 0.85c |
| $T_4$ | 130.4b | 152.0b | 0.84a | 0.69a | 3.0d | 3.3d | 0.59b | 0.57c |
| $T_7$ | 154.5b | 150.5b | 0.71b | 0.69a | 4.7c | 4.8 | 1.08b | 0.99c |

Means in the same column followed by the same letters are not significantly different at $P \leq (0.05)$ according to Duncans multiple range test.

Nostortium plants remarkably influenced by each of inoculation or intercropping (Table 3). Inoculated solid treatment increased seeds yield over un-inoculated solid treatment (traditional). The difference was significant in the first season only. $T_7$ and $T_3$ treatments were the best intercropping ones and gave the highest seeds yield. Seed index of nostortium plants did not varied by different studied treatments (Table 3). Inoculated solid treatments gave increases in N and P-contents in nostortium seeds than un-inoculated solid one. The differences were significant in both seasons. $T_7$ attained increase in N and P-contents higher than other intercropped ones (8.9 and 8.8 kg/fed.) for N and (1.87 and 2.13 kg/fed.) for P in first and second seasons respectively. The differences were mostly significant.

Data of Table (4) showed that inoculated solid treatment consistently increased fenugreek seeds yield over un-inoculated solid one. This means that inoculation may contribute in improving growth of intercropped plants and decrease competition between them. The best intercropping treatment was $T_3$ followed by $T_7$, whereas, they attained the highest seeds yield in both seasons. The highest value attained by $T_3$ in first season (872.9 kg/fed.). Seed index for fenugreek was not affected by all treatments. Inoculated solid treatment gave higher levels of N and P-contents than un-inoculated solid one. The differences were consistently significant. $T_3$ treatment was the best one within intercropped treatments. It attained the highest values of N and P-contents (20.3 and 2.03 kg/fed.) in first season and (30.2 and 1.04 kg/fed.) in second season respectively.

**Table (3):** Effect of intercropping nostortium plants with fenugreek plants on nostortium seeds yield (kg/fed.), seed index (g), N-and P-contents (kg/fed.), in the sercumestances of biofertilization.

| Treatment | Seeds yield (kg/fed.) | | Seed index (g) | | N-content (kg/fed.) | | P-content (kg/fed.) | |
|---|---|---|---|---|---|---|---|---|
| | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 |
| $T_1$ | 414.3b | 305.0a | 0.19a | 0.19a | 12.2b | 11.6b | 1.22bc | 0.92c |
| $T_2$ | 713.0a | 427.0a | 0.19a | 0.19a | 17.8a | 17.4a | 2.14a | 1.64b |
| $T_5$ | 147.3c | 158.2b | 0.19a | 0.19a | 6.3d | 6.5d | 0.74c | 0.71c |
| $T_6$ | 269.6b | 291.3ab | 0.19a | 0.20a | 7.5cd | 6.8d | 1.53ab | 1.47b |
| $T_7$ | 341.2ab | 387.0a | 0.21a | 0.20a | 8.9c | 8.8c | 1.87a | 2.13a |

Means in the same column followed by the same letters are not significantly different at P ≤ (0.05) according to Duncans multiple range test.

**Table (4):** Effect of intercropping mustrad and/or nonstortium plants with fenugreek plants on fenugreek seeds yield (kg/fed.), seed index (g), N-and P-contents (kg/fed.), under sercunestances of biofertilization.

| Treatment | Seeds yield (kg/fed.) | | Seed index (g) | | N-content (kg/fed.) | | P-content (kg/fed.) | |
|---|---|---|---|---|---|---|---|---|
| | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 |
| $T_1$ | 442.3b | 369.8c | 1.66a | 1.57a | 14.9c | 14.75cd | 1.27d | 0.95bc |
| $T_2$ | 685.5ab | 466.4a | 1.43c | 1.38a | 20.1ab | 19.5ab | 2.32a | 1.40a |
| $T_3$ | 872.9a | 488.0a | 1.53ab | 1.54a | 20.3a | 30.2a | 2.03b | 1.04b |
| $T_4$ | 652.9ab | 341.9c | 1.45bc | 1.32a | 20.7a | 17.0c | 1.72c | 0.86c |
| $T_5$ | 603.9ab | 374.4bc | 1.54a | 1.48a | 14.3c | 14.5cd | 1.64c | 1.0b |
| $T_6$ | 448.5b | 215.3d | 1.46abc | 1.34a | 10.0d | 10.4d | 1.21d | 0.57d |
| $T_7$ | 705.1ab | 353.3c | 1.45bc | 1.36a | 17.6b | 17.2c | 1.72c | 0.86c |

Means in the same column followed by the same letters are not significantly different at P ≤ (0.05) according to Duncans multiple range test.

Beneficial effect of intercropping non-legume plants on legume ones may be attributed to many factors like the transfer of $N_2$-fixed by legumes to non-legumes through direct decomposition of both nodules and fine roots (Abbas *et al.*, 1999). It may be useful in good distribution of the roots in soil profile which leads to decrease of leaching of mineral nutrients (Justus *et al.*, 1995) and consequently increase the use efficiency of these nutrients by intercropped plants. Also, symbiotic and non-symbiotic $N_2$-fixing microorganisms included by the inoculum produce growth phytohormones which potentially increase plant growth and development (Rodales *et al.*, 1999). In additon, microorganisms produce vitamins, amino acids, auxins, gebberellines and solubilize mineral nutrients (Sprenat, 1999) which reflected on growth stimulation of plants. In the present study, it was noted also that seeds of yield of legume crop (fenugreek) was potentially improved due to intercropping. This may be attributed to the good distribution of plant roots in soil profile and consquently the lower competition of nutrients in soil. Thus, inoculation of crops under intercropping systems with biofertilization may greatly improved growing environment of plants by increasing available nutrients (Bhatia *et al.*, 1998 and Singh and Kapoor, 1999), improving soil characteristics (Amora-Lazcano *et al.*, 1988 and Bethlenfalvay *et al.*, 1999) and decreasing pathogens around root zone. These factors together may lead to succession of intercropped plants under biofertilization circumstances.

The effectiveness of microbiol inoculation in increasing N and P-contents was confirmed by Fayez *et al.* (1985) and Gad (2001). Thus we can inform that microbial inoculation with biofertilizers especially in circumstances of intercropping is essential for improving growth of intercropped plants, which may decrease competition between them through improving hormonal and nutrition balance in the rhizosphere soil.

The effect of intercropping on N and P-uptaked by intercropped plants was studied by Abu-Taleb *et al.* (1999) and Abbas *et al.* (1999) and they reported results completely agreed with the result of the present study. It indicated that inoculation of intercropped crops is the reason of increasing nitrogen and phosphorus absorbance and assimilation by plant roots. Choose of intercropped crops must be based on the regular distribution of plant roots to decrease competition between them.

Treatments 3 and 7 attained largest value of LER, representing 1.73 and 1.92 in fist season and 1.34 and 1.97 in second season respectively (Table 5). All other treatments exhibited LER values over solid treatments by different degrees.This means that all studied intercropping systems induced increases in overall productivity of feddan, which make them of applicable value and this trend of research needs an additional efforts in near future. These results were in accordance with reports of (Keshta *et al.,* 2000 and Toaima *et al*., 2001).

**Table (5):** Effect of intercropping mustrad and nostortium plants with fenugreek plants on land equivalent ratio (LER).

| Treatment | 2002 | | | | 2003 | | | |
|---|---|---|---|---|---|---|---|---|
| | $L_f$ | $L_m$ | $L_n$ | LER | $L_f$ | $L_m$ | $L_n$ | LER |
| $T_3$ | 1.39 | 0.34 | -- | 1.73 | 1.05 | 0.29 | -- | 1.34 |
| $T_4$ | 1.04 | 0.27 | -- | 1.31 | 0.73 | 0.30 | -- | 1.03 |
| $T_5$ | 0.96 | -- | 0.21 | 1.17 | 0.80 | -- | 0.37 | 1.17 |
| $T_6$ | 0.71 | -- | 0.38 | 1.09 | 0.46 | -- | 0.68 | 1.14 |
| $T_7$ | 1.012 | 0.32 | 0.48 | 1.92 | 0.76 | 0.30 | 0.91 | 1.97 |

$L_f$ : Ratio of intercropped fenugreek seeds yield per solid fenugreek seeds yield.
$L_m$ : Ratio of intercropped mustard seeds yield per solid mustard seeds yield.
$L_n$ : Ratio of intercropped nostortium seeds yield per solid nostortium seeds yield.

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## الملخص العربي
## أثر بعض انظمة التحميل لبعض المحاصيل الطبية في وجود التسميد الحيوي

**محمد نور الدين[1] ، سامى ابراهيم يوسف[2]**
[1] معهد بحوث الأراضي والمياه والبيئة ، ج.م.ع ، الجيزة
[2] معمل النباتات الطبية والعطرية ، الدقى ، معهد بحوث البساتين ، الجيزة

أجريت تجربتين حقليتين بمنطقة النوبارية ، قرية الحسين ، موسمى ٢٠٠٢ ، ٢٠٠٣ بغرض معرفة تأثير تحميل الخردل و/أو حب الرشاد (كنباتات غير بقولية) على نبات الحلبة (بقولى) فى وجود التسميد الحيوى وذلك على الانتاجية من البذور وبعض المكونات الكيماوية لهذه المحاصيل.

اجريت عدة انظمة من التحميل ولقحت النباتات المحمله بلقاح مركب من بعض الميكروبات المثبته للازوت الجوى والاخرى المذيبه للفوسفات بالاضافة للتسميد العضوى بسبلة الدواجن ، بغرض تحسين ظروف التحميل. كانت افضل معاملات التحميل هما المعاملتان رقما ٧ ، ٣ (حلبه فى جانب الخط + حب الرشاد فى الجانب الاخر + خردل فى بطن الخط) ، (حلبه فى جابنى الخط + خردل فى بطن الخط) حيث اعطتا زيادة فى محصول الحلبة من البذور. وكلاهما كانتا الافضل انتاجية لبذور النباتات الاخرى (الخردل وحب الرشاد) عند المقارنة بباقى انظمة التحميل. أما عند مقارنة انظمة التحميل بمعاملتا النباتات الغير محمله (ملقحة او غير ملقحة) فقد اعطت معاملات التحميل انتاجية اقل من البذرو لكل من الخردل وحب الرشاد ، وذلك لان المساحة المنزرعة من أى من المحصولين داخل نظم التحميل تقل عن الفدان. وتظهر أهمية نظم تحميل نباتات غير بقولية على نبات بقولى من حساب معدل الكفاءة الأرضية والذى اظهر زيادة فى كل نظم التحميل كما حققت المعاملتان رقم ٧ ، ٣ أعلى قيمة كفاءة ارضية (١,٩٢ ، ١,٧٣) للموسم الاول و (١,٩٧ ، ١,٣٤) للموسم الثاني. أما وزن ١٠٠ بذرة فلم يتغير فى الغالب بسبب المعاملات المختلفة. زاد التحميل من محتوى البذور من النيتروجين والفوسفور وخصوصا المعاملتان ٧ ، ٣. كما زاد التسميد الحيوى وبصورة ثابتة تقريبا من محصول البذور ومن محتواها من النتروجين والفسفور مما يدل على ان التسميد الحيوى الميكروبي والعضوي يحسن من نمو هذه النباتات وبالتالى فقد يساعد على زيادة كفاءة نظم التحميل ونجاحها.