



MANAGEMENT OF *Fusarium oxysporum f. sp. rosae* USING METHAM SODIUM, DAZOMET AND BRASSICA BIOFUMIGANTS IN GREENHOUSE ROSE (*Rosa spp.*) PRODUCTION

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ABSTRACT

Alternative strategies to manage *Fusarium oxysporum f. sp. rosae* in greenhouse grown rose plants were investigated at Egerton University, Department of Horticulture Research and Teaching Field in 2005 and 2006. The treatments tested included; Dazomet, metham sodium and brassica biofumigants (*Brassica napus* and *Brassica juncea*). Dazomet and metham sodium were applied at 83.3g/m² and 0.12g/l to 1m² rates, respectively. *Brassica napus* and *Brassica juncea* were uprooted at the initial flowering stage and chopped into small pieces separately. The chopped brassicas were applied to the respective plots at the rate of 2, 3 and 4 kg/m². The results of this experiment showed that, Dazomet and metham sodium were effective in managing *Fusarium oxysporum f. sp. rosae*. However their activity was quite inconsistent and short lived. Reinfestation occurred soon after field aeration. *Brassica juncea* at an application rate of 3 and 4 kg suppressed *Fusarium oxysporum f. sp. rosae* more than *Brassica napus*. *Brassica juncea* at 3 and 4 kg had a similar effect as dazomet and metham sodium in the management of *Fusarium oxysporum f. sp. rosae*. Although metham sodium was the most effective against *Fusarium oxysporum*, the Brassica biofumigants especially *B. juncea* at 3 kg offers a better alternative for the management of *Fusarium oxysporum f. sp. rosae* in greenhouse rose production.

Keywords: *Fusarium oxysporum*, biofumigants, dazomet, metham sodium, rose plants.

INTRODUCTION

Low yields in many crops have been attributed to soilborne pests and pathogens. For example, the effect of *Fusarium oxysporum* on gladiolus (Mishra *et al.*, 2000) and on carnations (Ouellete *et al.*, 1999) is well documented. *Fusarium* survives in the soil for a long time (Blok and Bollen, 1996). The fungi enter the plant system through roots (Agrios, 1997), secreting pectolytic enzyme that catalyses the hydrolytic reactions responsible for the destruction of the middle lamellae of the xylem parenchyma. Wilting occurs due to plugging of the vascular elements by the gel contents (Daniel *et al.*, 1984). However, more common symptoms include; vein clearing, yellowing of the lower leaves, defoliation and finally death of the plant (Goncalves and Barreto, 2001). In more advanced stages of the disease, dark sap exudations have been observed to flow out of bark cracks. Orange, pink sporodochia with masses of conidia develop in bark cracks and lenticels (Skarmoutsou and Skarmoutsou, 1999).

The biofumigation potential differs from one species of brassica to the other (Angus *et al.*, 1994). Bioassay of volatiles emitted from hydrolysed tissue of various isothiocyanate producing brassica plants revealed widely varying toxicity effects (Matthiessen and Shackleton, 2000). In a study to compare the glucosinolate profiles in different brassicas, *Brassica juncea* was found superior to *Brassica napus* in inhibiting *Fusarium spp.* (Kirkegaard and Sarwar, 1998). The fungitoxic activity of pure glucosinolates enzymatic degradation products varies widely on poisoned media and in vapour phase, depending on their side chain chemical and physical properties.

Glucosinolate degradation products have been found to exert suppressive or control effect on a wide

range of soil borne pathogens including wheat take-all fungus (*Gaeumnomycetes graminis* var. *tritici*), *Meloidogyne spp.*, *Rhizoctonia solani* and *Fusarium oxysporum* (Warton *et al.*, 2001).

In another study, Verticillium wilt (*V. dahliae*) was successfully managed in a multiple year field study by incorporating broccoli residues into infested soil (Koike and Subbarao, 2000). However, in some cases, use of organic materials, an isolate of *Trichoderma harzianum*, a mycorrhiza and mixture of these failed to control *Fusarium oxysporum f. sp. Dianthi* (Bernal-Sierra and Arbelaez-Torres, 2000).

Dazomet efficacy requires uniform incorporation to a depth appropriate for the target pest, and crop to be protected (Juzwik *et al.*, 1997). For example, root diseases caused by *Fusarium spp.* and *Verticillium spp.* require treatment depth of 30 cm at an application rate of 425 kg/ha (Juzwik *et al.*, 1997). The efficacy of the fumigant increases with an increase in soil temperature. The fumigant effect has been reported to reduce in presence of organic particles (Juzwik *et al.*, 1997).

According to studies carried out by Sinha and Mukhopadhyaya (1993), dazomet is effective as nematicide and herbicide but not as fungicide. Dazomet has been found to be effective in controlling *Fusarium oxysporum f. sp. basilica* in combination with soil solarisation. The use of dazomet alone at different rates (25, 40, 50 or 75 g a.i / m²) was found to be ineffective.

Minuto *et al.*, (2000) evaluated the effectiveness of metham sodium and methyl bromide at full and half dose against *Rhizoctonia solani*, *Fusarium oxysporum f. sp. basilici* and *Colletotricum gloeosporioides*. The fumigants were found effective at full dose. However, the



two fumigants at reduced rates only resulted in an effective control of tested pathogen in combination with solarisation. Metham sodium is reported to be as effective as methyl bromide in controlling *Rhizoctonia solani* in *Thanatephorus cucumeris* (Marouli *et al.*, 2002). Combining metham sodium with short solarisation was found by Eshel *et al.*, (2000) to be more effective than either treatment alone in controlling *Sclerotium rolfsii* and *Fusarium oxysporum f. sp. basilici*. This demonstrated the synergistic effect of combining the treatments.

In a study involving the interaction of soil solarisation and metham sodium in the destruction of *Verticillium dhaliae* and *F. o. f. sp. Vasinfectum*, the fumigant dose required to kill 50% of the microsclerotia was 4 times higher at 25°C than at 35°C. Metham sodium however, has been found to kill more propagules than solarisation alone (Ben-Yephet *et al.*, 1988). In another field trial to control *Fusarium oxysporum f. sp. dianthi*, it was observed that disease incidence was 100% in untreated control, and 16% and 10% with 150 ml/m² and 300 ml/m² doses of metham sodium, respectively (Reuven *et al.*, 2000).

MATERIAL AND METHODS

Experimental site

The research was conducted at Egerton University horticultural research and teaching field in 2005 and 2006. The field is located at altitude 0°23 South, longitude 35°35 East and 2,225 m above sea level. The area receives moderate, mean annual rainfall of 1012 mm, mean maximum temperature of 22°C with minimum night temperature range of 5 to 10°C. Soil texture is sandy loam with pH of 5.5 - 6.0.

Experimental design and field layout

The experiment was laid down in randomized complete block design (RCBD) replicated three times.

Eight treatments were used in the study as indicated below,

Brassica napus 3 kg (BN 3),	Brassica juncea 2 kg (BJ 2)
Brassica napus 2 kg (BN 2),	Brassica napus 4 kg (BN 4)
Brassica juncea 3 kg (BJ 3),	Dazomet (DZ)
Brassica juncea 4 kg (BJ 4),	Metham sodium (MES)

Establishment of biofumigants and treatment application

The seeds of brasicca biofumigants (*Brassica napus* and *Brassica juncea*) used were obtained from Kenya Agricultural Research Institute (KARI). *Brassica napus* variety R3245 was planted on three beds 14 days earlier than *B. juncea* variety 3228 due to difference in time to maturity. Planting was done by direct seed drilling at inter-row spacing of 30 cm. The excess seedlings were uprooted upon germination to achieve intra-row spacing of 3 cm. Fertilizer application followed Kirkegaard and Sarwar

(1999) specifications of 20 kg N/ha, 20 kg P/ha and 18 kg S/ha. The brassicas were ready for use at initial flowering stage, which varied from 35 to 40 days for *Brassica juncea* and 52 and 54 days for *Brassica napus*. The treatments consistent of dazomet and metham sodium applied at 83.3g/m² and 0.12g/l per 1m² rates, respectively as well as *Brassica napus* and *Brassica juncea* applied at 2, 3 and 4kg/m².

Dazomet was thoroughly incorporated into the soil at the rate of 83.3g/m² to a depth of 30 cm. Metham sodium at the rate of 0.12g/l to 1m² was diluted in 5 liters of water and applied using a watering can. The application rates were as per the product specification. The plots were covered with clear polyethylene of 0.14 mm thickness. The polyethylene edges were buried 15 cm into the soil to ensure airtight conditions for three weeks.

The brassica plants were carefully uprooted at initial flowering stage and chopped into small pieces of about 1 cm. The chopped pieces of each brassica variety were immediately applied to respective plots at the rate of 2, 3 and 4 kg/m². The material was incorporated into the soil at about 30 cm depth.

The pathogen isolation and inoculum preparation followed Gonzalez-Torres *et al.*, (1993) procedure with some modification. *Fusarium oxysporum* spores were determined by counting the colony forming units on cultured media, on monthly basis. This involved use of soil dilution plate technique as described in laboratory manual for fusarium research (Lester *et al.*, 1994). The chemical fumigants were applied as per the product specification. The plots were covered with clear polyethylene (0.14 ml thick). The polyethylene edges were buried 15 cm into the soil to ensure airtight conditions for three weeks.

Data analysis

Analysis of field data was done with the Mixed Models procedure of SAS V9.1 statistical package (SAS Institute, 2002). The UNIVARIATE procedure of SAS was used to check that the data were normally distributed before analysis.

RESULTS AND DISCUSSIONS

The score of *Fusarium oxysporum* colony forming units reflected the effect of the treatments on this pathogen. The colony forming unit (CFU) varied with the treatments and the season. *Brassica napus* at 3 and 4 kg/m² significantly ($p \leq 0.05$) reduced the CFU by an average of 18% compared to the control. This level of control was significantly equal in strength to the 46% reduction in CFU observed in plots treated with metham sodium in season one and both the fumigants in season 2. However, the efficacy of methan sodium is quite unpredictable. It is likely to change from one type of soil to the other under different environmental conditions. For example, under high temperature conditions dazomet was more effective as a fungicide than metham sodium and vice versa Gan *et al.*, (1999). In this research, the difference in the environmental conditions is likely to have



had an effect on the efficacy of these fumigants. Average maximum greenhouse temperature was 35°C in the first season and 39°C in the second season. Therefore it is evident that during hot weather conditions dazomet is effective as a fumigant compared to cold weather.

The results of this study further confirm the antifungal effect of brassica biofumigants to suppress soilborne pathogens. The variations that exist in the toxicity of isothiocyanates (ITCS) are also shown as different results were obtained from different *Brassica* spp. The efficacy differed between the brassica species and rate of application. *B. juncea* at an application rate of 4 kg had a similar effect as the chemical fumigants used (Table-1). *B. napus* at all rates of application (2, 3 and 4kgs) and *B. juncea* at 2 kg showed some positive control effect, though not significantly different from the plot with no treatment.

Brassica juncea was therefore superior to *B. napus* in its suppressive effects against against *F. oxysporum* f. sp. *rosae* which supports the findings of Dunne *et al.*, (2003) and August *et al.*, (1994) who reported that roots of Canola and Indian mustard released compounds which inhibited the growth of *Gaeumannomyces graminis* var. *tritici* (Ggt) and that Indian mustard roots appeared to be more effective than Canola roots in inhibiting Ggt growth. *B. napus* even at higher application rate could not match the suppression effect achieved with *B. juncea*. This might be attributed to the low concentration of methyl isothiocyanate in *B. napus*. Previous studies have shown the property of ITCs for inhibiting fungal growth, but have not established whether the ITC concentrations achievable in the soil from the breakdown of brassica tissue are sufficient to control pathogenic fungi (August *et al.*, 1994). It is therefore possible that the use of higher quantity chopped material of *B. napus* may have a positive effect. Much documentation supports the concept that volatiles emitted from hydrolysed tissue of various isothiocyanate producing brassica plants vary in their toxicity effect (Matthiessen and Shackleton, 2000).

The results obtained with the brassica biofumigants were stable for both seasons (Table-1). In the second *B. juncea* was very effective in the management of the fungus compared to the chemical fumigants.

Plots treated with metham sodium maintained low levels of CFU for a longer period of four months compared to other treatments in season one. However, it was observed that after this period, the CFU started increasing in number (Figure-1a). This trend was also seen in the second season. These observation shows that the use of dazomet, metham sodium, *Brassica juncea* and *B. napus* at 4 kgs to manage *F. oxysporum* f. sp. *rosae* is effective for the first four months after soil fumigation.

While metham sodium was found to be a good fumigant as well a fungicide (Ammati and Nyambo, 1998), Kim *et al.*, (1994) observed that the efficacy of fumigant is quite inconsistent. It varies with changes in weather and soil characteristics as observed in this research.

The effectiveness of metham sodium could also be attributed to the method of application. Drip irrigation as a method of chemigation has been found to improve its efficacy (Browne *et al.*, 2002). The use of the Watering Can to apply the chemical in this study is likely to have increased its efficacy. This method of application ensured proper coverage of the experimental plots. However, the effectiveness of metham sodium may require application at suitable temperature, which has not yet been established. For example, high greenhouse temperature that rapidly accelerates the release of methyl isothiocyanate (MITC) impacts negatively on the efficacy of metham sodium, while low temperature limits the amount of MITC released (Brown *et al.*, 1991).

The formulation of dazomet in powder form may not effectively penetrate the soil particles to suppress the pathogens totally. The fungus therefore, increased rapidly on the treated plots after aeration. It is possible that the fungal spores that escaped control during fumigation were very vigorous in sporulation hence a rapid increase in CFU count.

Table-1. Average number of *Fusarium oxysporum* colony forming units analyzed per 1g of artificially infested soil.

Treatments	Season 1	Season 2
Control	5.6 a*	1.4 bc
BN 2 kg	4.8 ab	1.7 ab
BN 4 kg	4.5 ab	1.4 bc
BN 3 kg	4.9 ab	2.0 a
BJ 2 kg	4.9 ab	1.4 bc
BJ 3 kg	4.2 bc	1.7 b
BJ 4 kg	3.7 bc	1.4 bc
DZ	3.8 bc	1.0 c
MEZ	3.0 c	1.7 ab

*Means within a column followed by different letters are significantly different at $P \leq 5\%$ level of significance according to Duncan Multiple Range Test. The abbreviations used in the Table stands for *Brassica napus* (BN), *Brassica juncea* (BJ), Dazomet (DZ) and Metham sodium (MES). The treatments were applied m⁻² of an area.

The roses, which were treated with chemicals and biofumigants, showed variation in growth. Roses from plots treated with *B. juncea* at the rate of 3 or 4 kg/m² had longer stems compared to those stems harvested on plots with other treatments in the first harvest in both seasons (Table-2). Stems of rose plants attained length of 70 cm, which is good length for export market. However, the length of stems obtained from plots treated with different rates of *B. napus* and *B. juncea* at 2 kg were not significantly different.

Shorter stems of roses were obtained from plots treated with chemical fumigants, (dazomet and metham



sodium) and were of the same length as those from untreated plots. The use of metham sodium as a fumigant does not increase plant growth (Utkhede and Smith, 1993).

The second harvest was affected by reinfestation of soil pathogens leading to shorter stems (Table-2). Ten percent reduction in stem height was observed from the second harvest of season two compared to the first harvest of the same season. There was a perfect negative relationship between plant performance and increase in root galls and score of *F. oxysporum* colony forming units. An increase in root galls and CFU resulted into a decrease in the growth parameters studied. Fumigation of rose field prior to planting is therefore a very necessary practice. However stem length of flowers harvested from plots treated with three different rates of *B. napus*, *B. juncea* at 2 kg and Metham sodium were not significantly different. Poor plant performance was evident with dazomet as a treatment. Plots treated with dazomet had plants with the shortest stems ($p \leq 0.05$) compared to plots with no treatment in both seasons. This supports the findings of Thingstrup *et al.*, (1998), who observed reduced shoot growth of flux in plots fumigated with dazomet. The cause of weaker growth could be linked to the effect of the fumigant on the arbuscular mycorrhizal fungi (AMF). Thingstrup *et al.*, (1998) noted that soil fumigation with dazomet totally prevented AMF colonisation throughout the experiment and therefore ascribing to the suppression of mycorrhizal formation that is essential for plant growth.

Effect of the biofumigants and chemicals on the stem and flower diameter of roses

The quality of roses (stem diameter and size of the bloom) observed in this experiment varied from one treatment to the other. *Brassica juncea* at 3 and 4 kg gave the best stem quality in terms of thickness. Average stem and flower diameter from plots treated with *B. napus* at 4 kg/m², metham sodium, *B. juncea* 2 kg and *B. napus* 3 kg were statistically similar. Dazomet and plots with no treatment produced plants with the weakest stems. The stems were 20% shorter compared to the best stems harvested on the plots treated with *B. juncea* 3 or 4 kg (Table-3). The size of rose blooms was not significantly different across all treatments, except for *B. napus* at 2 and 3 kg and the control plot.

In the second harvest stem diameter on all plots treated with biofumigants except brassicas at 2 kg were statistically similar. *B. juncea* at 2 kg, Dazomet and plots with no treatment had the weakest stems. This trend was observed with the size of rose blooms harvested (Table-3).

It was also observed that stem thickness declined by almost 50% in the second harvest compared to the first harvest (Tables 3 and 4). However the change in bloom sizes between the first and second harvest was only 18% less.

Stem diameter was more affected than the flowers in the second season (Table-4). The stems were very thin; this could be attributed to the variation in weather. During this period the greenhouse temperature was high (36°C-38°C) with low relative humidity than that required for proper growth of roses.

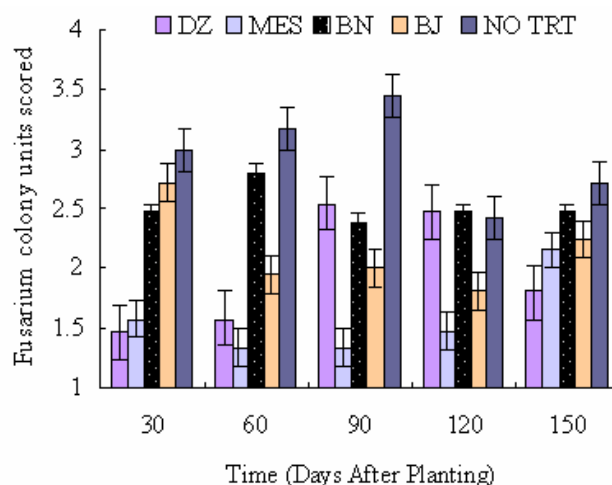


Figure-1a. Monthly trend of *Fusarium* colony units scored (season 1). Vertical line on each bar represents \pm S.E.

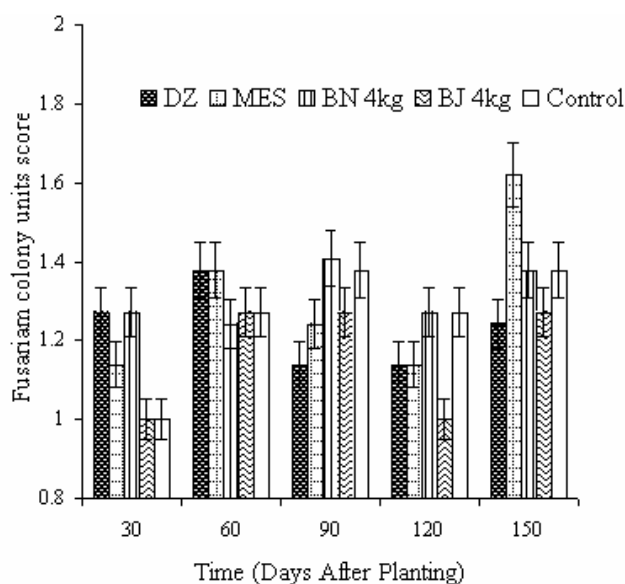


Figure-1b. Monthly trend of *Fusarium* colony units scored (Season 2). Vertical line on each bar represents \pm S.E. influence of brassica biofumigants and chemicals on selected growth parameters of roses.

**Table-2.** Influence of brassica biofumigants, dazomet and metham sodium on stem length (cm) of rose.

Treatments	Season one		Season two	
	Harvesting times		Harvesting times	
	1 st	2 nd	1 st	2 nd
NO TRT	53.07c ¹	42.63d	50.80c	48.07cd
BJ 2 kg	59.60bc	43.17d	53.03c	51.77abc
BJ 3 kg	73.50a	62.57a	69.37a	49.67bcd
BJ 4 kg	70.70ab	57.27ab	61.30abc	55.43a
BN 2 kg	58.40bc	55.63abc	58.10bc	53.17abc
BN 3 kg	58.87bc	55.70abc	61.83abc	52.53abc
BN 4 kg	60.57bc	55.93bcd	66.67ab	53.53ab
DZ	56.40c	47.27cd	54.37c	45.43d
MES	59.63bc	55.03abc	58.10bc	52.37abc

¹Means followed by different letters are significantly different at 5% level of significance according to Duncan's Multiple Range Test. The abbreviations used in the table stands for *Brassica juncea* (BJ), *Brassica napus* (BN), Dazomet (DZ) and metham sodium (MES). The treatments were applied per m² of an area.

Table-3. Effect of chemicals and brassica biofumigants on stem and flower diameter (Season 1).

Treatments	First harvest		Second harvest	
	Flower diameter	Flower diameter	Flower diameter	Flower diameter
	(cm)	(cm)	(cm)	(cm)
BJ 3 kg	0.89a ¹	3.91a	0.48a	3.24a
BJ 4 kg	0.80ab	3.90a	0.41abc	3.16a
BN 4 kg	0.72bc	3.44a	0.40abc	3.03abc
MES	0.72bc	3.64a	0.42ab	3.23a
BJ 2 kg	0.70bc	3.25a	0.33c	2.73bc
BN 3 kg	0.68bc	3.09ab	0.44a	3.35a
BN 2 kg	0.66c	3.11ab	0.41abc	3.12ab
DZ	0.65c	3.40a	0.34bc	2.99abc
NO TRT	0.61c	2.41b	0.33c	2.63c

¹Means followed by different letters are significantly different at 5% level of significance according to Duncan's Multiple Range Test. The abbreviations used in the Table stands for *Brassica juncea* (BJ), *Brassica napus* (BN), Dazomet (DZ) and metham sodium (MES). The treatments were applied per m² of an area.

**Table-4.** Effect of the chemicals and biofumigants on stem and flower diameter (Season 2).

	First harvest		Second harvest	
	Stem (cm)	Flower (cm)	Stem (cm)	Flower (cm)
BJ 2kg	0.63cd	2.63cd	0.33c	2.73bc
BJ 3 kg	0.80ab	3.77ab	0.48a	3.24a
BJ 4 kg	0.90a	4.00a	0.41abc	3.16a
BN 2 kg	0.73bc	3.30abc	0.41abc	3.12ab
BN 3kg	0.63cd	2.60cd	0.44a	3.35a
BN 4kg	0.67bcd	3.13bc	0.40abc	3.03abc
DZ	0.63cd ¹	3.13bc	0.34bc	2.99abc
MES	0.70bcd	3.30abc	0.42ab	3.23a
Control	0.57d	1.93d	0.33c	2.63c

¹Means followed by different letters are significantly different at 5% level of significance according to Duncan's Multiple Range Test. The abbreviations used in the Table stands for *Brassica juncea* (BJ), *Brassica napus* (BN), Dazomet (DZ) and metham sodium (MES). The treatments were applied per m² of an area.

CONCLUSIONS

The use of *Brassica napus* at 3 and 4 kg/m² was effective in the management of *Fusarium oxysporum f. sp. rosae* in greenhouse grown rose plants the biofumigant achieved a reduction level of Colony Forming Unit counts that was significantly equal in strength to the 46% reduction observed in plots treated with metham sodium. Greenhouse producers of rose plants can therefore use *Brassica napus* biofumigants as alternative to the synthetic fumigants and realize reduction in *Fusarium* attack and enhanced flower quality.

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