



Scan to know paper details and
author's profile

The Potential of Root Exuded Secondary Metabolites of *Tagetes* for Controlling Root-knot Nematode (*Meloidogyne Javanica*) on Vegetable Crops (Brinjal and Chilli)

Abhilasha Srivastava & Lalit Kumar

ABSTRACT

Root exudates of marigold (*Tagetes erecta*), collected from intact live plants via newly developed root exudates trapping system after proper fractionation gives rise to a total of five allelofractions viz., C, B, A, E and D of different polarity (non-polar to polar). The obtained fractions were found enriched with one single compound in each of them. Two Emulsifiable Concentrate (EC) from non-polar fraction (A&B) and one from medium polar fraction viz., C) developed from 90% pure fractions, clearly revealed the role of polarity, exposure time and the concentration of isolated fractions in imparting their toxicity against second stage juveniles (J2) of *Meloidogyne javanica* in laboratory experiment. EC formulations of fractions A and B (soluble in Hexane & ethyl acetate) was found 100 % toxic to the juveniles at 200 $\mu\text{g ml}^{-1}$ of concentrations after an incubation period of 24 h whereas, EC formulation developed from fractions C (acetone soluble) showed nearly 70 % juvenile mortality at 700 $\mu\text{g ml}^{-1}$ concentrations, at an incubation period of 72 h. The EW formulation of fraction D & E (polar) could not observe to produce any mortality in juveniles in all their test concentrations ranged from 800-1200 $\mu\text{g ml}^{-1}$ hence not utilized for nematode control.

Keywords: allelochemicals, allelopathy, *meloidogyne javanica*, root exudates, *tagetes erecta*.

Classification: FOR code: 070399

Language: English



London
Journals Press

LJP Copyright ID: 925662
Print ISSN: 2631-8490
Online ISSN: 2631-8504

London Journal of Research in Science: Natural and Formal

Volume 19 | Issue 7 | Compilation 1.0



© 2019. Abhilasha Srivastava & Lalit Kumar. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncom-mercial 4.0 Unported License (<http://creativecommons.org/licenses/by-nc/4.0/>), permitting all noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

The Potential of Root Exuded Secondary Metabolites of *Tagetes* for Controlling Root-knot Nematode (*Meloidogyne Javanica*) on Vegetable Crops (Brinjal and Chilli)

Abhilasha Srivastava^α & Lalit Kumar^σ

ABSTRACT

Root exudates of marigold (*Tagetes erecta*), collected from intact live plants via newly developed root exudates trapping system after proper fractionation gives rise to a total of five allelofractions viz., C, B, A, E and D of different polarity (non-polar to polar). The obtained fractions were found enriched with one single compound in each of them. Two Emulsifiable Concentrate (EC) from non-polar fraction (A&B) and one from medium polar fraction viz., C) developed from 90% pure fractions, clearly revealed the role of polarity, exposure time and the concentration of isolated fractions in imparting their toxicity against second stage juveniles (J₂) of *Meloidogyne javanica* in laboratory experiment. EC formulations of fractions A and B (soluble in Hexane & ethyl acetate) was found 100 % toxic to the juveniles at 200 µg ml⁻¹ of concentrations after an incubation period of 24 h whereas, EC formulation developed from fractions C (acetone soluble) showed nearly 70 % juvenile mortality at 700 µg ml⁻¹ concentrations, at an incubation period of 72 h. The EW formulation of fraction D & E (polar) could not observe to produce any mortality in juveniles in all their test concentrations ranged from 800-1200 µg ml⁻¹ hence not utilized for nematode control. Non-polar fraction based EC formulations (A & B) at their higher concentrations of 150 & 200 µg ml⁻¹, were not only found extremely effective in juvenile mortality but also retarded egg hatch of the test

nematode up to an extent of more than 98 %. At higher concentrations, the said formulations were also observed to cause permanent damage to the exposed eggs as there was no further hatching noticed when treated egg masses were kept in ordinary water for their revivals. As compare to control, the most active EC formulations of non-polar and medium polar fractions viz., A, B & C in pot experiments conducted to control nematode infestation in brinjal and chilli were found to reduce 30-40% and 20-30% gall formation respectively. Apart from gall control the formulations were also found capable in crop bust and yield gain. In case of brinjal more than 100% yield gain was observed in some of the treatments whereas, in case of chilli nearly 40% yield gain was observed.

Author^α: Scientist (WOS-B), Department of Science & Technology, New Delhi.

σ: Principal Scientist, Indian Institute of Pulses Research, Kanpur.

Keywords: allelochemicals, allelopathy, *meloidogyne javanica*, root exudates, *tagetes erecta*.

I. INTRODUCTION

Plant-parasitic nematodes are known to cause a great damage to agricultural and horticultural crops. Especially in vegetable crops nematode infestation is considered to be a prime constraint in realizing optimum yield potential. Root-knot nematodes (*Meloidogyne* species) infect almost all types of vegetable plants and may cause

considerable damage. On an estimate global crop loss caused by plant parasitic nematodes is more than \$100 billion per annum with an average yield loss of 15-20 % as reported in several cash crops (Koenning *et al.*, 1999, Sasser and Freckman, 1987). Cultivation of resistant varieties, cultural practices such as crop rotation, cover cropping, green manuring, organic amendments, chemical nematicides etc. have been advocated and subsequently being utilized for management of nematode infestation below economic threshold levels in various crops (Eapen *et al.*, 2005). Though the application of conventional nematicidal chemicals had an initial dramatic impact but the exorbitant cost of these chemicals, their non-availability, effect on non-target organisms, induction of resistance in target species and side by side several kind of negative impact on environment etc. have proved a shattering blow to their utility potential (Taylor, 2000). Other conventional methods too have their own technical and operational limitations of one kind or the others. Since most of the management practices have their own limitations and not yielded desirable results hence need was always felt to develop some of the alternative control options. In this context the use of botanical pesticides is now being emerging as an effective alternative to protect crops (Haseeb *et al.*, 1981&1984, Prot *et al.*, 1983, Adekunle *et al.*, 2003). Among the botanicals, *Azadirachta*, *Eucalyptus*, *Chrommelina*, *Sida acuta* and *Tagetes* have been found to be very effective in nematode control (Umar *et al.*, 2010). As far as marigold is concern it can suppress 14 genera of plant parasitic nematodes by including root knot nematodes (*meloidogyne* spp.). Apart from this, in recent days, interest has also been shifted in discovering nematostatic compounds of the plant origin (Chitwood, 2002). In nature, plants produce a number of secondary metabolites to defend themselves against various pests, diseases and nematodes as the exudates of numerous plants or extracts of their various parts were reported to contain nematicidal or nematostatic compounds (Devine and Jones, 2003; Rodger *et al.*, 2003; Ruhm *et al.*, 2003). In this respect,

marigold (*Tagetes* spp.) is considered an excellent material owing to be of its tremendous allelopathic potential against Plant Parasitic Nematodes. Different genera of marigold plant (*Tagetes* spp.) were not only evaluated for their tremendous capability to reduce nematode population in fields (Khan and Siddiqui, 2001; Reynolds *et al.*, 2000; Walia and Gupta, 1997) but the various extracts prepared from different plant parts were also observed toxic to juveniles in laboratory experiments (Sasanelli and Addabbo, 1993; Hassan *et al.*, 2003, Saravanapriya and Shivakumar, 2005; Natarajan *et al.*, 2006). Though most of the studies of this plant mainly remained focus either on the field observations or the different extracts based preparations except few where the thienyls in the roots and several terpenoides in the essential oil of leaves and flowers (Padma *et al.*, 1997) were demonstrated to be the toxic chemical principals against *Meloidogyne species* but the compounds released by plants via their secondary metabolic procedures through their roots as exudates remained unexplored. These kinds of chemical compounds may be more potent killer or inhibitor for nematodes and thus can easily be exploited as natural nematicides. Further generated information may also be utilized as a lead for development of more potent and environmental friendly nematicides either by taking the structure of most active isolated chemical directly or by utilizing them as a lead. Therefore, with this view a study was undertaken to isolate root exudates from intact live plants of marigold and to assess the effect of isolated chemicals against second stage juveniles and egg hatch of *Meloidogyne javanica* in laboratory and subsequently in pots on brinjal and chilli crops.

II. MATERIAL AND METHODS

2.1 Root Exudates Collection and Fractionation

A root exudates trapping system as suggested by Kumar, 2004, Kumar *et al.*, 2008 was employed to collect root exudates of intact live plants of marigold. As shown in fig. 1 the system comprises

of separating funnel (field with soil) and conical flask (filled with distilled water). The marigold plant was planted in soil of conical flask. After 15 days of plantation the root penetrates the soil of funnel and entered the distilled water of conical flask. Water from conical was removed weekly and immediately replaced with fresh one. Approximately hundred liters of root zone water was collected periodically from 20 plants of marigold grown till maturity (90days) in 20 sets of root exudates trapping systems. Initially, the entire collected root zone water was slowly reduced to 15-20 % of the original volume by pouring it in sufficient number of petriplates and allowing evaporating under continuously moving fan. The concentrated root zone water obtained so was transferred to a separating funnel and then partitioned with equal amount of ethyl acetate. In this process allelocompounds got fractionated into two major groups *viz.*, polar (water layer) and non – polar (ethyl acetate layer). Different allelocompounds present in both of the layers were recovered in five fractions *viz.*, A, B, C, D & E of distinct polarity by fractionally crystallizing the layers in suitable polarity of solvents as per the fractionation scheme depicted in Fig. 2.

2.3 Preparation of Formulations and Test Concentrations

Obtained all the five fractions *viz.*, A, B, C, D and E were converted to suitable emulsifiable concentrate (EC) formulations of 10% in order to achieved uniform solubility. The emulsification of fractions was achieved by combining the isolated crystallized products with pesticide dispersible liquid carriers and with carrier vehicle assistants, ie emulsifying agent and/or dispersing agents. The choice of formulations auxiliaries is determined as per the nature and polarity of extracted fractions. Therefore, non-polar fractions *viz.*, A, B and C were converted to 10% EC formulations by using the tween-80 (10%) as emulsifier and cyclohexanone as a dispersible liquid carriers whereas, polar fractions D and E were emulsify to EW formulations by using water and tween-80 (10%) respectively as dispersible

liquid carrier and emulsifying agents. Emulsifications in both kinds of the recipes were achieved by vigorously agitating the mixtures at high speed and at $45 \pm 2^\circ\text{C}$ for an hour. Test solutions of different concentrations *viz.*, 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 and $1200 \mu\text{g ml}^{-1}$ were prepared by taking the appropriate amount of EC.

2.4 Bioassay

Egg masses of the root knot nematode (*Meloidogyne javanica*) were collected from the infected brinjal roots and further cultured on brinjal plants to maintain regular population of the juveniles during experimentation. Experiments were conducted on freshly hatched second stage juveniles in six different dilutions in 50-mm diameter petriplates of approximately 15-ml capacity. Initially 9 ml distilled water was poured in each test petriplates then required quantity of test EC formulation was added in accurate amount to get the desire concentration in final volume of 10 ml and mixed well. One ml of freshly hatched second stage juveniles of *M. javanica* suspension containing approximately 300-400 in number was added to each petriplate and kept at $25 \pm 1^\circ\text{C}$ for 24, 48 and 72 h. All the treatments were replicated thrice along with a set of control containing formulation auxiliary's *viz.*, cyclohexanone and tween-80 similarly prepared. After specified period of incubation, the treated suspension in petriplates was stirred properly and one-ml suspension was transferred to another petriplate. It was diluted 10 times with distilled water to reduce the concentration of chemical much below to its toxic level to observe the possibility of revival if any. Observations on live and dead nematodes were recorded after 24 h of dilution by using stereoscope microscope. Dead nematodes appeared straight, while live nematodes retained the characteristic sigmoid shape and exhibited movement (Sethi and Prasad, 1962). The per cent mortality was worked out from the average of three replications in each case and converted to natural mortality according to Abbots formula. Effect of most active formulation was also observed on egg hatch of *Meloidogyne*

javanica. Replicated bioassay tests for egg hatch were conducted by using different concentrations *viz.*, 50, 100, 150 and 200 $\mu\text{g ml}^{-1}$ of test EC formulation. Five egg masses were kept in each petriplate. A separate set of three petriplates containing five egg masses in each with formulation auxiliaries was kept as control. Observations on egg hatch were recorded after incubation period of 1, 3, 5, 7, 9, 11, 13, 15 and 18 days.

2.4 Pot ZExperiments

Pot culture experiments for both of the crops i.e. brinjal and chilli were conducted in big size pots of 20 inches diameter during March to June. The pots were filled by taking approximately 12-15 kg of ordinary soil belongs to Indo-Gangatic Plain. It is a sandy loam texture soil of taxonomical class *Typic Ustrtochrept* and was found to retain physicochemical properties as PH- 7.5, organic carbon 0.50%, available N, P & K 150, 250, 200 Kg/h. Three to four seeds of brinjal (Pusa-long) and chilli (G-5, a local cultivar) were sown separately in each pot where after germination plants were thinned to one I per pot. After 10 days of germination the soil of pots was initially incubated with freshly hatched second stage juveniles of *M. javanica* @ 500 per pot approximately. After 48 hr of incubation the pots were treated with different concentrations *viz.*, 0.750, 1.00, 1.25 g/ pot of root exuded chemical fractions EC formulations. The chemicals were applied to the surface of pots after dissolving the appropriate quantity of EC formulations in 1 liter of water. Whereas, one set of pots for each crop was kept as control where no chemical treatment was made. All treatments were replicated thrice. The pots were arranged in a completely randomized design in the greenhouse at $25 \pm 5^\circ\text{C}$ for nearly 90 days. Sufficient moisture in pots was maintained by applying irrigation as and when required. Observations on the plant characters *viz.*, shoot length, shoot weight, root length and root weight, gall index etc and yield & yield attributing parameters were taken at the time of termination of experiment. Root-knot

index was assessed on 0-5 scale (Sasser *et al.*, 1984).

2.5 Data Collection and Statistical Analysis

After termination of the experiments the plants of both of the crops were gently removed from the pots. The shoots were counted and excised from the roots. The lengths of shoots and roots were measured with a ruler. The shoots and roots of individual plants were weighed with an electric balance. The galls and egg masses on the whole root systems were counted under a stereoscope at a magnification of $40\times$. The obtained data's from both of the experiments *viz.*, lab and pot were statistically analyzed by using SPSS software. And the differences between treatments were determined by LSD 5% probability level.

III. RESULTS

3.1 Recovery of Allelo Compounds

By repeatedly following the proposed fractionation scheme the allelochemicals were successfully fractionated into five different group of compounds of distinct polarity *viz* A, B, C (non – polar *ie.*, organic layer), D and E (polar, aqueous layer) with more than 90% purity as a single component as checked by TLC and HPLC. Three compounds *ie.*, A, B and C with R_F values 0.86, 0.616 and 0.313 were detected on TLC as major component of non-polar fraction *ie.*, ethyl acetate layer. Whereas, three compounds under the R_F zones *viz.*, 0.86 & 0.527 (acetone: methanol 65: 35) C & D and 0.360 (methanol: water 80: 20) E were detected clearly in polar fraction (water layer). During fractional crystallization of ethyl acetate layer in hexane, compound B and C were crystallized and hence recovered by filtration. Compound A remained soluble in hexane phase (filtrate) was recovered by evaporation as brown color viscous liquid at room temperature. Both B and C were successfully separated out from each other by using chromatographic columns. Rest of the two compounds *viz.*, D(R_F 0.360) and E (R_F 0.527) present in water layer were recovered and

separated out by following the repeated steps of fractionation and column chromatography. Compound E was found completely soluble in methanol and water whereas, compound D was found completely soluble in water only. Approximately, a total of 10 grams products by comprising approximately of three grams of white crystalline product of non-polar nature (ethyl acetate soluble) containing both A and B and 7 grams of polar compounds viz., C, D and E was recovered from the entire collected root exudate water (approximately 100 liters) of 20 plants of marigold, grown throughout the period of three months in 20 sets of root exudates trapping system.

3.2 Effect on 2nd stage juveniles of Root knot Nematode

Table 1 shows the effect of different concentrations of treatment on the *M. javanica* juvenile mortality. The nematicidal activity of all the five formulations viz., A, B, C, D and E of marigold root exudates varied according to the nature i.e., the polarity of the isolated fractions, doses and the incubation period. Formulations, those developed especially from the non-polar chemical fractions viz., A and B was found to exhibit severe impact on the mortality and paralyzes of second stage juveniles of *Meloidogyne javanica* in comparison to the formulations developed from medium polar fraction (C). Maximum juvenile mortality was achieved with the formulation developed from highly non-polar fractions i.e. A and B followed by the formulations developed from C. Since, formulation D and E could not produced any mortality in juveniles up to maximum concentration of 1200 $\mu\text{g ml}^{-1}$ hence rejected for further test. Non-polar fraction based EC formulations (A&B) were found detrimental to the juveniles at extremely low concentrations ranged from 50-200 $\mu\text{g ml}^{-1}$. The activity of both of the formulation was found highly correlative with time of exposure and the concentration of the formulations. At lowest concentration (50 $\mu\text{g ml}^{-1}$), compare to control, though both of the formulations, gives rise only 7-10 % mortality in

juveniles after an exposure period of 24 h but there was a progressive and highly significant increase in the mortality with increase in exposure period. Therefore, both of the formulations at same concentration resulted approximately about 20 % nematode mortality after exposure periods of 48 and 72 hrs. Quick and absolute mortality of the same formulations were observed at their highest concentration viz., 200 $\mu\text{g ml}^{-1}$ at this concentration of formulations, not a single nematode was found to survive even after 24h of exposure period. Formulation of C developed from medium polarity of fraction also exhibited significant impact on the mortality of *M. javanica* juveniles. The formulations were observed toxic with different capabilities between the concentrations ranged from 100 to 700 $\mu\text{g ml}^{-1}$. This formulation brings about nearly 55% juvenile mortality at 700 $\mu\text{g ml}^{-1}$ concentrations after an exposure periods of 72 h, over control.

3.3 Effect on Egg Hatch

Apart from the juvenile's mortality, the non polar fraction based E C formulations (A & B) were also observed extremely effective in retarding the egg hatch properties of the test nematode species. Results, as compare to control, revealed a grave impact of the test formulations on egg hatch in all the test concentrations of the formulation ranging from 50-200 $\mu\text{g ml}^{-1}$ (fig 3). In case of control egg hatch started immediately after first day of treatment which increased progressively with time and reached to the maximum within 15 days. But in case of treatments of both of the formulations egg hatch was found retarded to a grave extent and could not complete up to 18 days of exposure. Since the retardation effect of test formulations was found concentration dependent hence a positive linear correlation between reductions in egg hatch with the concentration of formulation was observed. In comparison to hatching in control, approximately 55 and 80 % reduction, in egg hatch was noticed at 50 and 100 $\mu\text{g ml}^{-1}$ concentrations of the formulation. Whereas, at higher concentrations viz., 150 & 200 $\mu\text{g ml}^{-1}$ both of the formulations were observed extremely effective in retarding egg hatch of the

test nematode up to an extent of more than 98 %. Though the test formulations at their lower concentrations of 50 and 100 $\mu\text{g ml}^{-1}$ found to retard egg hatch of test nematode only when the nematodes continuously remained under exposure of chemical but as and when the treated egg masses were transferred to distilled water magnitude of egg hatching increase. In contrary, at higher concentration of the formulations ie 150 $\mu\text{g ml}^{-1}$ and above the treated eggs received permanent damage as there was no further hatching noticed even after transferring the egg masses in distilled water for longer period.

3.4 Effect on Gall Formation and Yield

The effectiveness of prepared E C formulations of tagetes root exuded chemicals was studied in big pots by using sandy clay loam soil as filler on two vegetable crops i.e. brinjal and chilli after their soil application. The parameters studied in this experiment were plant height, root length, number of galls/plant, yield/plant and average yield/h etc. The data presented in Tables 2 (brinjal) and 3 (chilli) clearly revealed that different concentrations (0.750g/pot, 1g/pot, 1.25g/pot) of prepared formulations of fractions A, B & C not only brought about significant reduction in root-knot development caused by *M. javanica* but also observed to gain in root and shoot length, branch numbers etc. thereby a highly significant increase in yield of both of the crop plants was observed. As compare to control, a significant improvement in plant phenological parameters viz., plant height, root length, number of branches etc of both of the crop plants have been observed in almost all treatments of prepared E C formulations however the differences in these parameters was not found significant neither with the treatments concentrations (0.750, 1.00, 1.250g/pot) nor with the type of fractionated formulation. As far as root-knot development is concern, in case of brinjal crop the maximum reduction nearly to 35% in root-knot development was observed at highest dose (1.25g/pot) of formulations derived from completely non-polar fractions (A&B). However, at same concentration the medium

polarity fraction based formulation (C) also caused root-knot development reduction nearly to 27%. Same kind of results were also observed in case of chilli for this crop all the formulations were found capable in reduction of gall formation up to an extent of 25-35% at their highest concentration (1.25g/pot). The nematode gall reduction on roots of both of the crop was found directly correlated to enhance the yield of the crops to a large extent. In case of brinjal the yield gain per plant as well as per hectare was found maximum (approximately 90%) in treatments of the formulations those derived from fraction A and B at their highest concentration i.e.1.250g/pot however medium polarity fraction based E C formulation (C) also observed quite efficacious by gaining a yield advantage nearly to 20% . In case of chilli all the three kind of formulations viz., A, B and C were observed almost equally effective in gaining yield advantage. The formulations at their highest concentrations (1.250) were found to enhance chilli yield to 35-40%.

IV. DISCUSSION

Our results clearly demonstrated the effect of root exuded chemicals of marigold extracted from intact live plants on overall development of *Meloidogyne javanica*. The chemicals at their low concentrations were not only found absolute fatal to the larvae of this nematode but also inhibited the hatching and permanently damage the egg. The chemicals were not only found active in vitro conditions but also observed quite efficacious in reducing the nematode gall formation and yield gain in brinjal and chilli crop at pot conditions. The finding altogether suggest that the chemicals secreted by roots of tagetes have great capability not to reduce the nematode population in field if this crop is grown as an intercrop or in rotation but also be used as potent bio-nematicide against *Meloidogyne* spp. nematodes. These results are in agreement with those of previous authors (Hackney and Dickerson, 1975; Alam *et al.*, 1977) who reported nemato-toxic properties of Tagetes spp. The effect of isolated chemicals on egg hatch

is also in good agreement of several other co-workers for example, Siddiqui and Alam, 1988 proposed that root exudates of *T. minuta* inhibited nematode egg hatch. However, egg hatch of *P. penetrans* in root diffusate of *T. patula* was comparable to that from a good host plant (Pudasaini *et al.*, 2008), even though *T. patula* was reported to be antagonistic to *P. penetrans* (Evenhuis *et al.*, 2004; Pudasaini *et al.*, 2008). A similar mechanism was reported for the soybean cyst nematode, *Heterodera glycines* (Ichinohe) and Italian ryegrass, *Lolium multiflorum*, which as a non-host increased egg hatching, resulting in a depletion of the lipid reserves of the hatched juveniles (Riga *et al.*, 2001). Moreover very recently the effect of root exudates of linseed crop on egg hatch of *Meloidogyne javanica* was also reported by us (Kumar *et al.*, 2011). As far as the chemistry of root exudates of marigold is concern the role and identity of allelopathic compounds has not been fully characterized but Gommers and Bakker, 1988 hypothesized that α -terthienyl was a major component. More recently, El-Gengaihi *et al.*, (2001) isolated three nematicidal compounds *viz.*, 5 (-ent-1-ol)-2,2-bithienyl, sigma-4, 22-dien-3-beta-ol, and 5-(4-acetoxy-1-butenyl)-2,2-bithienyl from the chloroform extract of aerial parts of *T. erecta*, *T. patula*, and *T. minuta*. Though all these chemicals are reported in various kinds of extracts of different plant parts which can be considered primary compounds of plant. But as far as the root exuded chemicals of intact live plant is concern, release compounds may either be chemically different or may be the distinct derivatives of already reported chemicals since they release by intact live plants via their secondary metabolic reactions. Thus our current research findings can be considered as confirmative study to hypothesis that nematodes are killed after entering the root system or coming into contact with soil that contains marigold's bioactive compounds released by plant during their life cycle. However to make the finding more valuable chemical structure elucidation of isolated compounds is required.

REFERENCES

1. Adekunle, O. K. and Fawole, B. 2003. Comparison of Effects of Extracts of Siam weed, Neem and Carbofuran on Generation time and Reproduction of *Meloidogyne incognita* Race 2 on Tomato. *Environment and Ecology* 21(3): 720-726.
2. Alam, M. M., Saxena, S.K. and Khan, A. M. 1977. Influence of inter-culture of marigold and margosa with some veg- etable crops on plant growth and nematode population. *Acta Bot. indica* 5 : 33-39.
3. Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. *Annual Review of Phytopathology* 40: 221-249.
4. Devine, K.J. and Jones, P.W. 2003. Investigations into the chemo attraction of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* towards fractionated potato root leachates. *Nematology* 5: 65-75.
5. El-Gengaihi, S.E., Osman, H.A., Youssef, M.M.A. and Mohamed, S.M. 2001. Efficacy of *Tagetes species* extracts on the mortality of the reniform nematode, *Rotylenchulus reniformis*. *Bull N.R.C. Egypt* 26: Pp 441-450.
6. Eapen, S. J., Beena, B. and Ramana, K. V. 2005. Tropical soil microflora of spicebased cropping systems as potential antagonists of root-knot nematodes. *Journal of Invertebrate Pathology* 88: 218-225.
7. Evenhuis, A, Korthals, G.W. and Molendijk, L.P.G. 2004. *Tagetes patula* as an effective catch crop for long-term control of *Pratylenchus penetrans*. *Nematology* 6: 877-881.
8. Gommers, F.J. and Bakker, J. 1988. Physiological changes induced by plant responses or products. In Diseases of Nematodes G.O. Poinar Jr., H.-B. Jansson (Ed..) *The Boca Raton Press CRC* 1: 3-22.
9. Hachney, R. W. and Dickerson, O. J. 1975. Marigold, castor bean, and chrysanthemum as controls of *Meloidogyne incognita* and *Pratylenchus alleni*. *J. Nematol* 7: 84-90.
10. Haseeb, A., Khan, A. M. and Saxena, S. K. 1981. Effect of certain alkaloid bearing plants

- on the mortality and larval hatching of *Meloidogyne incognita*. *Geobios* 8: 3-5.
11. Haseeb, A., Siddiqui, M. A. and Alam, M. M. 1984. Toxicity of latex bearing plants to phytonematodes. In *Environ and Biotic Interact*, A.K. Dattagupta and R.P. Maleyvar (Ed.). *Kurukshetra University Press Kurukshetra*: 67-71.
 12. Hassan, S.M.E., Rahman, M.S., Kabir, M.A., Hasan, M.R. and Sarker, M.G.M. 2003. Effect of some plant extracts on the root-knot nematode (*Meloidogyne javanica*) of lady's finger. *Agriculture Science Digest* 23: 63-64.
 13. Khan, A.A. and Siddiqui, M.A. 2001. Evaluation of nematicidal properties of *Azadirachta indica*, *Tagetes patula*, *Ficus racemosa* and *Nerium indicum* against *Meloidogyne incognita* attacking tomato. *Bionotes* 3: 82.
 14. Koenning, S.R., Overstreet, C., Noling, J.W., Donald, P.A., Becker, J.O. and Fortnum, B.A. 1999. Survey of Crop Losses in Response to Phytoparasitic Nematodes in the United States for 1994. *Suppl. Journal of Nematol.* 31: 587-618.
 15. Kumar, L. 2004. IIPR technique for the extraction, separation and purification of allelo compounds from plant species. In: *Proceedings of International Workshop on Protocols and Methodologies in Allelopathy CSKHPKV Agriculture University Palampur, H P., India*: 34-42.
 16. Kumar, L., Singh, B. and Srivastava, G.K. 2011. Effect of root exuded allelochemicals of linseed on egg hatch and mortality of juveniles of root-knot nematodes (*Meloidogyne javanica*). *Journal of Food Legumes* 24: 123-127.
 17. Kumar, L. and Varshney, J.G. 2008. Efficacy of sesame (*Sesamum indicum*) root exudates against major weeds of pulse crops. *The Indian Journal of Agricultural Sciences* 78 : 842-847.
 18. Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S. and Dhakshnamoorthy, G. 2006. Cold aqueous extracts of African marigold, *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. *Crop Protection* 25: 1210-1213.
 19. Oostenbrink, M. 1960. *Tagetes patula* L. al svoorvrucht van ' enkele land- en tuinbouwgewassen op zand-en dalgrond. *Meded. LandbHoogesch. OpzoekStns Gent* 25: 1065-1075:
 20. Padma, V., Suman, K., Satyawati, S., Vasudevan, P., Kashyap, S. and Sharma, S. 1997. *Tagetes*: a multipurpose plant. *Bioresource Technology* 62: 29-35.
 21. Prot, J. C. and Kornpbrost, J. M. 1983. Effect of *Azadirachta indica*, *Hannoa undulate* and *H. klaineana* seed extracts on the ability of *Meloidogyne javanica* juveniles to penetrate tomato roots. *Review of Nematology* 6: 330-332.
 22. Pudasaini, M.P., Viaene, N. and Moens, M. 2006. Effect of marigold (*Tagetes patula*) on population dynamics of *Pratylenchus penetrans* in a field. *Nematology*: 8: 477-484.
 23. Pudasaini, M.P., Viaene, N. and Moens, M. 2008. Hatching of the root-lesion nematode, *Pratylenchus penetrans*, under the influence of temperature and host. *Nematology* 10: 47-54.
 24. Reynolds, L.B, Potter, J.B. and Ball- Coelho, B.R. 2000. Crop rotation with *Tagetes* sp. is an alternative to chemical fumigation for control of root-lesion nematodes. *Agronomy Journal* 92: 957-966.
 25. Riga, E., Welacky, T., Potter, J., Anderson, T., Topp, E. and Tenuta, A. 2001. The impact of plant residues on the soybean cyst nematodes, *Heterodera glycines*. *Canadian Journal of Plant Pathology* 23: 168-173.
 26. Rodger, S., Bengough, A.G., Griffiths, B.S., Shebbs, V. and Young, J.M. 2003. Does the presence of detected root border cells of *Zea mays* after the activity of the pathogenic nematode *Meloidogyne incognita*? *Phytopathology* 93: 1111-1114.
 27. Ruhm, R., Dietsche, E., Harloff, H.J., Lieb, M., Franke, S. and Anumann, J. 2003. Characterisation and partial purification of a white mustard kairomone that attracts the

- beet cyst nematode, *Heterodera schachtii*. *Nematology* 5: 17-22.
28. Saravanapriya, B. and Shivakumar, M. 2005. HPLC analysis of different plant extracts exhibiting nematocidal activity. *Indian Journal of Nematology* 35: 76-77.
29. Sasanelli, N. and D-Addabbo, T. 1993. Effect of *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* leaf and root extracts on Italian populations of *Meloidogyne species* *Nematologia Mediterranea* 21: 21-25.
30. Sasser, J.N. and Freckman, D.W. 1987. Vistas on Nematology: a commemoration of the twenty fifth, anniversary of the society of nematologists. J. A. Veech and D.W. Dickson (Ed.). *Society of Nematologist, Inc. Hyattsville*, 7-14.
31. Sasser, J.N. and Freckman, D.W. 1986. A world perspective on Nematology; in Vistas on Nematology. Veech, J. A. and Dickson, D.W.(Ed.) *Society of Nematologist. Maryland: 7-14*.
32. Sethi, C.L. and Prasad, D. 1982. In vitro toxicity of DBCP to some phytophagous nematodes and hatchability of *Heterodera cajani*. *Indian Journal of Nematology* 12: 65-72.
33. Siddiqui, M.A. and Alam, M.M. 1988. Studies on the nemato-toxicity of root exudates of certain species of *Tagetes*. *Indian Journal of Nematology* 18: 335-337.
34. Taylor, R.W.D. 2000. Methyl bromide – is phase out on schedule? *Pesticide Outlook* 11: 54-57.
35. Umar, I., Muhammad, Z. and Okusanya, B.A.O. 2010. Effect of organic amendments on the control of *Meloidogyne javanica* (Kofoid and White, 1919) Chitwood, 1949) on Tomato (*Lycopersicon lycopersicum*, Mill). *Agri, Business and Tech. J.* 8: 63-67.
36. Walia, K.K. and Gupta, D.C. 1997. Management of root-knot nematode. *Meloidogyne Javanica* on vegetable crops with *Tagetes sp.* *Indian Journal of Nematology* 27: 18-23.



Fig.1: Marigold plant grown in root exudates trapping system

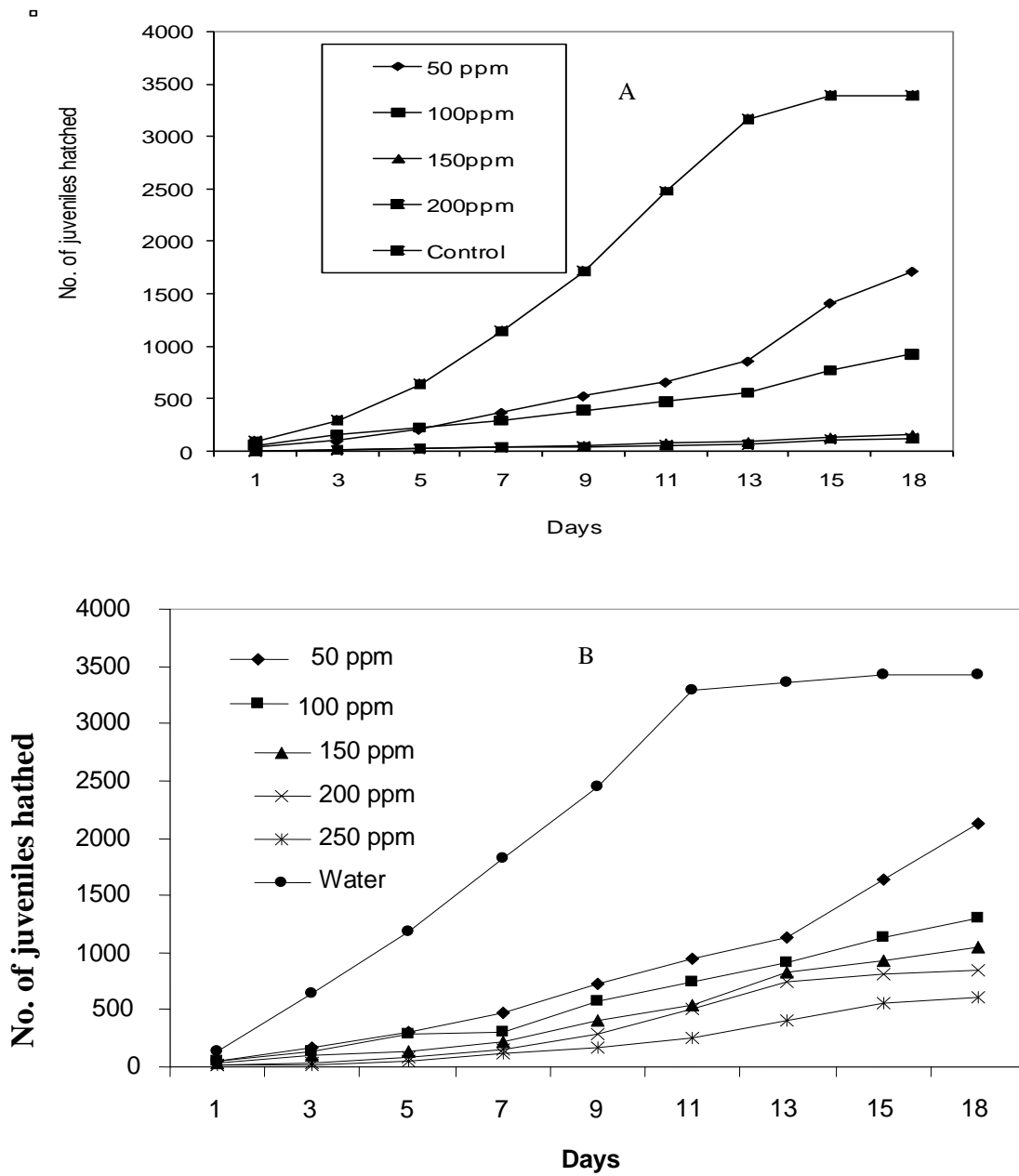


Fig. 3: Effect of formulation A and B on egg hatch of *Meloidogyne javanica*

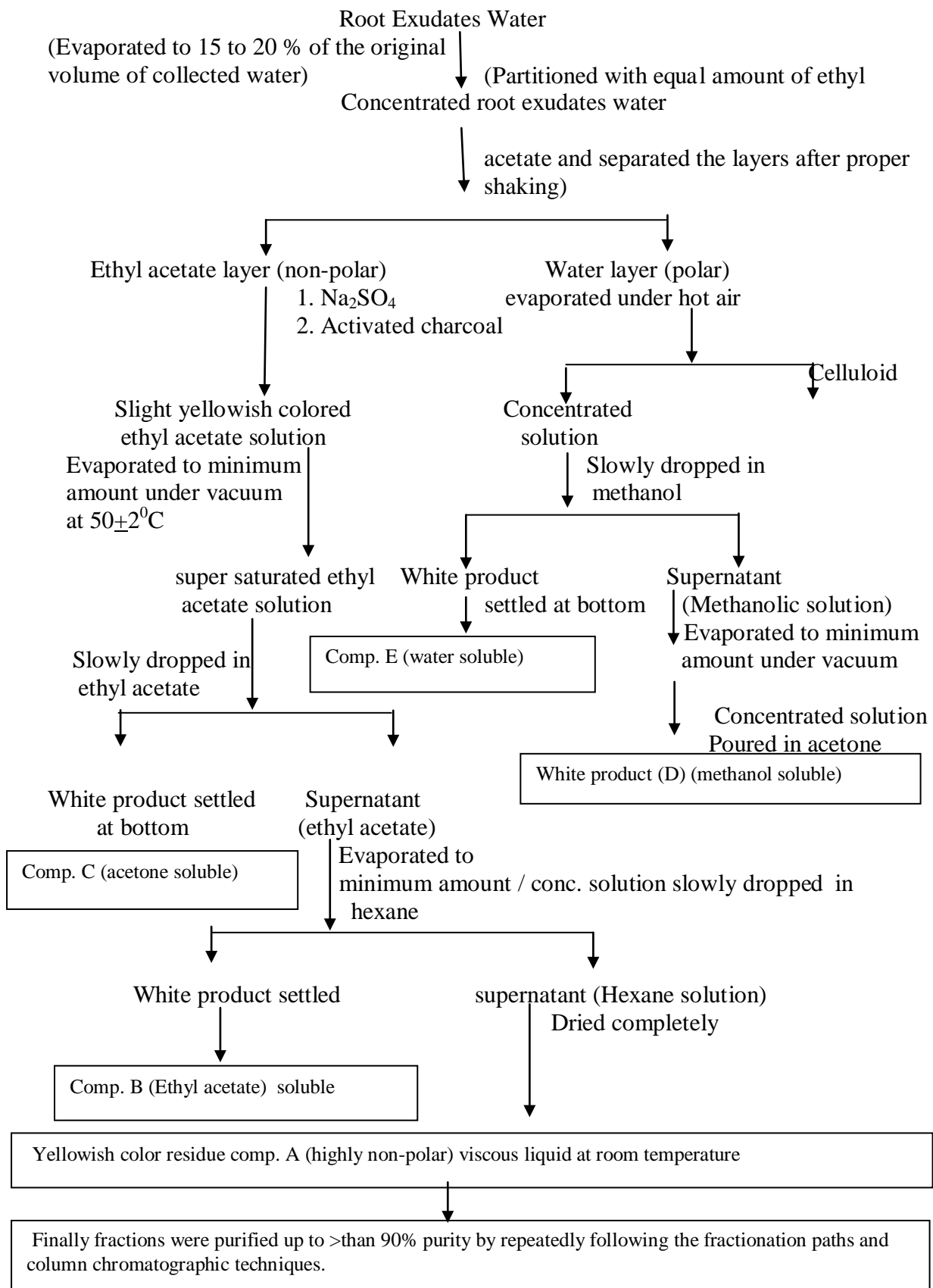


Fig. 2: Extraction Procedures Adopted to Extract the Allelopathic Compounds of Marigold Root Exudates

Table 1: Effect of different polarity fractions of marigold root exudates on the mortality of second stage juveniles (J2) of *Meloidogyne javanica* at different concentrations and time exposures

Exposure time (hr.)	Concentrations												
	50	100	150	200	250	300	500	600	700	800	900	1000	Mean
Effect of non-polar fraction (hexane soluble)													
24	3(9.4)	24(29.3)	30(33.2)	45(42.1)	95(76.9)	100(90.0)							49.4(46.8)
48	9(17.0)	32(34.2)	36(36.9)	52(46.2)	95(77.7)	100(90.0)							53.9(50.3)
72	18(25.0)	41(39.8)	49(44.2)	65(53.8)	99(87.3)	100(90.0)							62.0(56.7)
Mean	10(17.1)	32(34.4)	38(38.1)	54(47.3)	96(80.6)	100(90.0)							
CD (P=0.05), Time=1.62, Concentration=2.29, Time X Concentration=3.97													
Effect of non-polar fraction (soluble in dichloromethane)													
24	0(1.9)	10(17.8)	21(27.1)	29(32.7)	92(73.9)	98(84.0)							42(39.6)
48	7(15.6)	16(23.2)	27(31.5)	36(36.9)	93(75.1)	99(87.3)							46(44.9)
72	15(23.0)	30(33.2)	41(39.6)	54(47.1)	96(79.1)	100(90.0)							56(52.0)
Mean	8(13.5)	18(24.7)	30(32.7)	40(38.9)	94(76.0)	99(87.1)							
CD (P=0.05), Time=2.36, Concentration=3.34, Time X Concentration=5.78													
Effect of non-polar fraction (soluble in ethyl acetate)													
24	0(0.0)	7(15.0)	19(25.6)	35(36.1)	70(57.0)	79(62.7)							35(32.7)
48	2(6.6)	13(21.1)	24(29.1)	45(41.9)	83(66.0)	88(69.9)							42(39.1)
72	4(11.9)	27(31.5)	38(37.8)	58(49.8)	89(70.4)	91(72.9)							51(45.7)
Mean	2(6.2)	16(22.5)	27(30.8)	46(42.6)	81(64.1)	86(68.5)							
CD (P=0.05), Time=1.61, Concentration=2.28, Time X Concentration=3.95													
Effect of medium polar fraction (soluble in acetone)													
24							13(21.3)	35(36.3)	42(40.4)	55(47.7)	68(55.6)	80(63.3)	49(44.1)
48							16(23.5)	40(39.4)	47(43.3)	59(50.2)	73(58.7)	85(67.7)	53(47.1)
72							20(26.5)	43(41.2)	53(46.5)	62(51.2)	77(61.1)	89(70.8)	57(49.7)
Mean							16(23.8)	40(38.9)	47(43.4)	59(50.0)	73(58.5)	85(67.3)	
CD (P=0.05), Time=1.70, Concentration=2.41, Time X Concentration=4.17													

(Figure in parenthesis is angular transform values)

Table 2: Effect of Tagetes Root Exudates Fraction on Growth and Yield Attributing Parameters of Brinjal Crop

Fraction	Dose/h	Plant Height (cm)	Root Length (cm)	Shoot Branch	Root Branch	Number Of Galls/ Plant	% Reduction Over Control	Average Yield (kg/pl.)	% Gain In Yield Over Control
Control	0.00	23	12	5	3	98	-	1.65	0.00
Hexane soluble	0.75	45	22	10	8	67	31.63	2.98	80.60
	1	46	25	11	8	63	35.72	3.25	96.97
	1.25	48	24	13	9	60	38.77	4.16	152.00
Ethyl acetate	0.75	42	19	10	7	67	31.63	2.75	60.67
	1	43	19	9	8	68	30.61	2.65	60.60
	1.25	44	21	11	7	64	34.69	3.15	90.90
Acetone	0.75	38	16	7	3	72	26.53	1.7	03.3
	1	38	17	8	3	75	23.47	1.7	03.3
	1.25	40	18	9	3	71	27.55	1.95	18.18
SEm±		3.85	2.43	1.25	0.68	5.47		0.25	
CV(%)		9.4	12.6	13.6	11.6	7.7		9.7	
CD(P=0.05)		11.4	7.2	3.7	2.0	16.1		0.74	

Table 3: Effect of Tagetus Root Exudates Fraction on Growth And Yield Attributing Parameters of Chilli Crop

Fraction	Dose/h	Plant Height (cm)	Shoot Branch	Number of galls/plant	% Reduction over control	Average Yield		% Gain in yield over control	
						Per plant (kg)	Per ha. (Tones)	Per plant	Per ha
Control	0.00	52	6	83	-	0.126	4.7	-	
Hexane soluble	0.75	77	7	70	15	0.176	5.6	40	19
	1.00	87	7	69	17	0.206	6.2	63	32
	1.25	88	8	61	27	0.215	6.4	70	36
Ethyl acetate	0.75	74	7	69	17	0.181	5.5	44	17
	1.00	76	7	63	24	0.204	5.9	62	26
	1.25	78	7	60	28	0.231	6.6	83	40
Acetone	0.75	70	7	63	24	0.166	5.1	35	09
	1.00	71	7	60	27	0.188	5.3	49	13
	1.25	71	7	54	35	0.24	6.9	90	47
SEm±		5.72	0.75	5.20		0.014	0.40		
CV (%)		7.7	10.8	8.0		7.1	7.0		
CD(P=0.05)		16.9	NS	15.3		0.041	1.2		

This page is intentionally left blank