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Review

Development of Indigenous *Cucumis* Technologies (ICTs) to alleviate the void created by the withdrawal of synthetic nematicides from the agro-chemical market

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The "Indigenous *Cucumis* Technologies" (ICTs) were researched and developed for the management of plant-parasitic nematodes, particularly *Meloidogyne* species, in an attempt to alleviate the void created by the withdrawal of synthetic nematicides from the agro-chemical markets and the drawbacks associated with the use of conventional organic matter as a nematode management practice. Currently, ICTs comprises of four technology types, namely (1) ground leaching, (2) nematode resistance, (3) inter-generic grafting and (4) fermented crude extracts. ICTs, in their various forms, consistently suppressed the nematode numbers and improved crop yields in experimental trials carried out in Limpopo Province, Republic of South Africa. The present paper reviews a decade of successful research and development in ICTs for the management of root-knot nematodes in low-input agricultural farming systems.

Key words: *Cucumis* species, fermented crude extract, ground leaching technology, inter-generic grafting, nematode resistance.

INTRODUCTION

Worldwide, the withdrawal of highly effective synthetic fumigants used in the management of plant-parasitic nematode populations has had economic consequences in many crop production systems (Mashela, 2007, 2002; Mashela et al., 2008). Global crop losses per annum caused by plant-parasitic nematodes have been estimated at 12% (Ferraz and Brown, 2002), whereas the South African estimate is 14% (Anon, 2011). In crops like watermelon (*Citrullus lanatus*), of which no genotypes resistant to root-knot nematodes (*Meloidogyne* species) are known, crop losses from 50% to complete crop failures had been reported (Barker et al., 1976; Lamberti, 1979; Oda et al., 1997). In economical terms, global annual crop losses due to damage by plant-parasitic nematodes have Been estimated at US $125 billion prior to the final withdrawal of methyl bromide from agro-chemical markets in 2005 (Chitwood, 2003).

After having been relied upon for over 50 years for suppressing population densities of plant-parasitic nematodes, the prohibition of methyl bromide fumigations left a serious void (Mashela, 2007; Mashela et al., 2008). The research and development of alternative strategies to replace methyl bromide have since become indispensable in crop health (Bello, 1998; Mafeo and Mashela, 2009a, b, 2007, 2002; Mashela et al., 2008; Pofu et al., 2009). Much work had been done to develop non-chemical and environment-friendly nematode management practices, such as the use of botanicals (Belo, 1998; Mashela, 2002, 2007; Mashela and Mpati, 2002; Mashela and Mphosi, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2008; Rajendran and Saritha, 2005; Sukul et al., 2001) and organic soil amendments (Nagesh and Reddy, 1997; Singh

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et al., 2001; Vedhera et al., 1998).

In South Africa, farmers have been arbitrarily classified into household, subsistence, emerging and commercial farmers in order to tailor-make and fast-track farmer-support services for each group (Anon, 2009). Household and subsistence farmers in marginal communities rely much on low-input agricultural systems, with an inter-cropping sequence comprising, almost always, maize (*Zea mays*), cowpea (*Vigna unguiculata*), watermelon (*C. lanatus*), pumpkin (*Cucurbita moschata*) and other minor crops. Due to this traditional "monocultural system" which is based more upon emotional than economical reasoning, the population build-up of *M*. species in this farming system, has since reached population levels that, in some cases, result into complete crop failure among components of the system (Fourie and Mc Donald, 2003). Incidentally, when water is available, tomato (*Lycopersicon esculentum*) and Swiss chard (*Beta vulgaris*) crops are grown during the off-season for the main intercrops. However, most of the tomato and Swiss chard cultivars available for use in Limpopo Province (Republic of South Africa) are highly susceptible to *M*. species, and therefore, further enhances the population build-up of nematodes. Due to the existence of *M. incognita* races 2 and 4, along with *M. javanica* (Kleynhans et al., 1996), breeding for nematode resistance for a small niche market like the one being described here, is uneconomical.

Traditionally, in low-input agriculture farming systems, farmers use organic amendments to suppress plant-parasitic nematodes and to provide essential nutrients (Mashela, 2002; Stirling, 1991). However, the use of conventional organic amendments to suppress plant-parasitic nematodes has drawbacks which include: (i) excessively large quantities (10 to 250 t/ha) of organic materials being required to obtain nematode suppression, (ii) high transport costs to haul the materials from the planting/collection site to the decomposition site and eventually to the field, (iii) long waiting periods for microbial decomposition, (iv) reduction in soil pH, and (v) often inconsistent results in nematode suppression (Mankau, 1968; Mankau and Minteer, 1962; McSorley and Gallaher, 1995a,b; Muller and Gooch, 1982; Rodriguez-Kabana, 1986; Stirling, 1991). Consequently, most marginal farming communities can hardly afford the use of conventional organic amendments in suppression of plant-parasitic nematodes.

The Indigenous *Cucumis* Technologies (ICTs) were researched and developed to address the plant-parasitic nematode problems in low-input agricultural systems, particularly for marginalised farming communities in South Africa. In particular, ICTs comprises of four technology types, *viz*. (1) ground leaching, (2) fermented crude extracts, (3) nematode resistance and (4) inter-generic grafting. Some of the components of the ICTs have been in existence for over 10 years with consistent results in nematode suppression and improved crop yield.

The aim of this paper is to review a decade of research and development in ICTs with the ultimate purpose of improving food security in marginal farming communities of the Republic of South Africa.

INDIGENOUS *CUCUMIS* SPECIES IN SOUTH AFRICA

The overview of indigenous *Cucumis* species in South Africa presented here is limited to (a) their pharmacological properties, (b) presence of potent chemicals, (c) attractiveness for use in nematode management, and (d) their minimum bioactive concentration against selected nematodes.

Pharmacological properties

The *Cucurbitaceae* family consists of 115 genera (Pitrat et al., 1999), most of which have been widely used for centuries in African traditional medicine (Rimington, 1938). South Africa is the centre of bio-diversity for wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*) (Kristkova et al., 2003). Crude extracts of *C. myriocarpus* derived from fruits and roots, along with the whole plant of *C. africanus,* contain pharmacological properties used in the treatment of liver damage, weakening of the immune system, lumps, jaundice, acute and chronic viral hepatitis, hepatic cirrhosis, persistent dyspepsia, epilepsy due to wind-phlegm, cancer, gonorrhea, boils and infections by intestinal roundworms (Anon., undated).

Presence of potent chemicals

Potent chemicals present in crude extracts of *Cucumis* fruits have been isolated and identified as cucurbitacins (Rimington, 1938). Plants belonging to *Cucurbitaceae* family contain a total of 12 cucurbitacin types, with cucurbitacin A in *C. myriocarpus* fruit and roots being the only one that is water soluble (Chen et al., 2005).

Cucurbitacins are oxygenated tetracyclic triterpenes with glycosides and originate from the mevalonic pathway (Inderjit and Malik, 2002). Cucurbitacins are used in plant defence against nematodes, fungi and insects (Inderjit and Malik, 2002; Inderjit et al., 1995; Mashela, 2002). Generally, cucurbitacin A, which had been widely investigated, comprises of two potent chemicals, namely, cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffrey, 1978; Rimington, 1938), which have more or less similarity to basic molecular structures of aldicarb ($C_7H_{14}N_2O_2S$) and fenamiphos ($C_{13}H_{22}NO_3PS$). Acute dermal toxicities (LD_{50}) of cucumin have been established for rat and rabbit as LD_{50} = 0.5 and 13 mg kg^{-1}, respectively, whereas those for aldicarb and fenamiphos are 5 and 9 mg kg^{-1}, respectively (Mashela, 2007).

Attractiveness for use in nematode management

Traditional practitioners orally-administered decoctions of crude extracts of *C. africanus* and *C. myriocarpus* fruits to control intestinal roundworms in humans *(Ascaris lumbricoides*), dogs (*Toxocara canis, Toxascaris leonine*), chickens (*Ascaridia galli*) and other domesticated animals (Pers. comm. of Limpopo Province Traditional Healers' Association). All these intestinal roundworms are zoo-parasitic nematodes (Mashela, 2007).

Motivated by these observations, Mashela and Mphosi (2002) opted to use crude extracts of *C. myriocarpus* fruits to suppress population levels of *Meloidogyne* spp. and the citrus nematode (*Tylenchulus semipenetrans*) in pot trials, with results showing at least 90% suppression of the nematodes. The technology, which came to be referred to as the ground leaching technology (GLT), involves the application of ground materials from selected plant organs in small quantities – the latter having both suppressive effect on nematodes and fertilizer effect on plants (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2010).

Minimum bioactive concentration

Bioactivity effects *in vitro* of crude extracts of *C. myriocarpus* fruit on *M. incognita* resulted in nematode mortalities from 87 to 95%, whereas the mortalities ranged from 83 to 96% on *T. semipenetrans* (Muedi et al., 2005). The minimum bioactive concentration of crude extracts from *C. myriocarpus* fruit for both *M. incognita* race 2 and *T. semipenetrans* was 7 µg/ml (Muedi et al., 2005).

GROUND LEACHING TECHNOLOGY

In GLT, mature fruits of *C. african*us and *C. myriocarpus* were cut into small pieces, dried at 52°C (Makkar, 1999) for 72 h and ground in a Wiley mill to pass through a 1-mm-pore sieve (Mashela, 2002). The material was applied soon after transplanting without first being subjected to microbial degradation (Mashela, 2002; Mashela and Mphosi, 2002). Crude extracts were spread in small quantities (2 to 5 g/plant) in a shallow hole around the base of the stem which translated to 20 to 71 kg ground material/ha for 4,000 tomato plants. The small quantities precluded high transport costs to haul the materials to the decomposition sites and then to the fields. Also, when used at transplanting, the waiting period for microbial decomposition was not necessary and the material hardly reduced soil pH (Mashela, 2007). Most importantly, suppression of nematode numbers was consistently achieved, regardless of the environment where the trials were conducted.

Originally, the fruit used for GLT were collected from the wild, but they were eventually collected from locally-propagated plants. The development of propagation protocols and fertilisation requirements of *C. myriocarpus* have been described by Mafeo (2005). Seeds of *C. myriocarpus* fruit have auto-allelopathic chemicals and leaching of allelochemicals is necessary for germination to occur (Mafeo and Mashela, 2009b). Moreover, Mafeo (2005) and Nkgapele et al. (2011a, b) demonstrated that moderate fertilisation was required for *C. africanus* and *C. myriocarpus* in order to achieve optimum growth and fruit yield.

Post-emergent application

Crude extracts of *C. myriocarpus* fruit suppressed the plant-parasitic nematodes in greenhouse and microplot trials by over 90% (Mashela, 2002; Mofokeng et al., 2004; Shakwane et al., 2004), and in field trials by over 80% (Mashela, 2007). Additionally, relative to untreated controls, the crude extracts increased soil electric conductivity from 95 to 160%, but had no significant effect on soil pH. Also, the material improved fruit yield and growth of tomato and dry shoot mass of citrus seedlings in various trials (Mashela, 2007; 2002; Mashela et al., 2008; Mphosi, 2004). The efficacy of crude extracts from *C. myriocarpus* fruit, when compared with that of aldicarb and fenamiphos suggested that the three materials did not have significant differences in nematode suppression and fertiliser effect (Mashela et al., 2008).

In GLT microbial decomposition had negligible role in the efficacy of crude extracts of *C. myriocarpus* fruit, as shown by lack of interactions between this material and *Bacillus* species on variables measured (Mphosi et al., 2004). Also, independence of GLT from microbial activity was demonstrated through elimination of *Bacillus* species in predictive stepwise regression models when using crude extracts of castor bean (*Ricinus communis*) fruit (Mashela and Nthangeni, 2002; Mofokeng et al., 2004) and fever tea (*Lippia javanica*) leaves (Ngobeni et al., 2004). However, microbial activity was required when crude extracts of cabbage (*Brassica oleracea*) leaves were used for suppression of *M. incognita* race 2 in tomato production in pot trials (Mangena, 2005). Shakwane et al. (2004) demonstrated that crude extracts of *C. myriocarpus* fruit promoted nodulation of *Bradyrhizobium japonicum* in cowpea (*V. unguiculata*) crops.

Pre-emergent application

Crop yield losses are generally proportional to initial population densities (Pi) of nematodes (Seinhorst, 1967). Ideally, a material in GLT system should serve as a pre-emergent bio-nematicide in order to keep Pi at the lowest level possible. The seed germination assays performed *in vitro* suggested that at 5 g/L tap water of crude extracts of *C. myriocarpus* fruit had allelopathic effects to tomato, watermelon and butternut squash seedlings (Mafeo and Mashela, 2009a), as was in the case of maize, finger millet, sorghum and onion (Mafeo and Mashela, 2009b). In greenhouse trials, the material completely inhibited seedling emergence of all dicotyledonous crops tested (Mafeo and Mashela, 2010).

Cucumin, derived from crude extracts of *C. myriocarpus* fruit, was shown to also suppress the division of cancer cells in animals (Van Wyk et al., 1997). However, the suppression occurred at dosages which were toxic to healthy cells, whereas at reduced dosages, the material stimulated division of cancer cells. Quadratic relationships between cell divisions and dosages ascribed to cucumin, characterised responses of biological systems to extrinsic factors, had been described as density-dependent growth patterns (Salisbury and Ross, 1992). Using the observations of stimulation animal cells as reference, dosages were reduced *in vitro* from 0 to 2.25 g/plant, with germination of tomato, watermelon and butternut squash having significant ($P \leq 0.01$) negative quadratic relationships with increasing dosages (Mafeo and Mashela, 2009b). In the trial, increasing dosages of crude extracts of *C. myriocarpus* fruit explained 91, 97 and 91% of the total treatment variation in stimulation of seed germination of tomato, watermelon and butternut squash plants, respectively. Results consistently showed that crude extracts of *C. myriocarpus* fruit at high dosages had allelopathic effect on seed germination of test plants. Therefore, the material was considered unsuitable for use as a pre-emergent bio-nematicide.

Confirmation studies were initiated using the Curve-Fitting Allelochemical Response Data (CARD) computer model (Liu et al., 2003) to establish dosages where crude extracts of *C. myriocarpus* fruit could stimulate and inhibit germination of various crops in order to estimate the pre-emergent quantities (Mafeo and Mashela, 2010; Mafeo et al., 2010). The CARD model provided six biological indices (Liu et al., 2003), with the total sum of transformations (k) serving as an indicator of the sensitivity of the test plant to the bio-nematicide (Mafeo et al., 2010). Generally, k is inversely proportional to the degree of sensitivity of the test plant to the material (Liu et al., 2003). Initially, the trials involved 18 different plant cultivars with 10 being dicotyledonous plants and eight monocotyledonous plants. The 10 dosages within the interval from 0 to 2.25 g were arranged in a randomised complete block design (RCBD) with five replicates. At the moment of harvesting, 18 days after initiating the treatment, the mean seedling height, root length, coleoptile length and hypocotyl diameter of each cultivars were separately subjected to the CARD Model (Mafeo and Mashela, 2010; Mafeo et al., 2010), which indicated that the 18 crops had different sensitivities (k values) to crude extracts of *C. myriocarpus* fruit, with clear stimulatory and inhibitory dosages (Mafeo and Mashela, 2010; Mafeo et al., 2010; Mafeo et al., 2011a, 2011b). Pre-emergent quantities for applying crude extracts of *C. myriocarpus* fruits using GLT were computed within the overall stimulated range, with validation suggesting that within this range there was no allelopathic effect of the materials on germination of the test crops.

Fermented crude extracts technology

GLT system is naturally labour-intensive and could, therefore, costly for large-scale commercial farmers. Development of bio-nematicide from fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits would enhance the application of GLT through irrigation water in commercial farming agriculture. However, since only cucurbitacin A in *C. myriocarpus* fruit was soluble in water (Chen et al., 2005), it was uncertain whether crude extracts of *C. africanus* fruit could also serve as fermented crude extracts in suppression of nematodes since cucurbitacin B is insoluble in water. Thus, fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits were tested separately and reduced nematode population densities by 89% (range 80 to 100%) and 69% (range 52 to 79%), respectively, with the reproductive factor (Pf/Pi) values being below one (Pelinganga et al., 2011). At low dilutions both materials had fertilizer effect on tomato plants, while at high dilutions each was phytotoxic. Results of the study (Pelinganga et al., 2011) demonstrated that the two materials could serve as potent bio-nematicides at low dilutions, in what we have since termed "botinemagation" - the application of botanicals for nematode suppression through irrigation water.

Nematode resistance technology

The two *Cucumis* species earlier mentioned could also be used as alternative crops for the production of traditional medicine and bio-nematicides. Preliminary studies showed that *Cucumis* species could be used as rootstocks for watermelon (*C. lanatus*) cultivars, all of which do not have resistant genotypes to *M.* species. Thus, host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *Meloidogyne* species were investigated in pot, microplot and field trials (Pofu et al., 2010a, b; 2009), where both *Cucumis* species were shown to be highly resistant to *M. incognita* races 2 and 4 and *M. javanica,* which are dominant in tropical and subtropical areas of South Africa with sand (Kleynhans et al., 1996).

However, the two *Cucumis* species were tolerant to the spiral nematode (*Helicotylenchus dihystera*) and the ring nematode (*Criconema mutabile*) under field conditions (Pofu et al., 2011a).

Mechanisms of resistance to *M.* species in two *Cucumis* species were investigated in a greenhouse trial (Pofu and Mashela, 2011). Generally, resistance in plant-parasitic nematodes had been broadly classified as pre-infectional and post-infectional with resistance manifesting prior and after penetration of roots, respectively (Kaplan and Keen, 1980). Establishment of resistance type in plant-parasitic nematodes is essential where germplasm could be introgressed into breeding lines since only post-infectional resistance is introgressible (Kaplan and Keen, 1980; Thurau et al., 2010). In *C. africanus* and *C. myriocarpus* there were pre-infectional and post-infectional resistance forms, respectively (Pofu et al., 2010a). Consequently, resistant germplasm in *C. myriocarpus* could be useful for introgression in highly nematode-susceptible genera such as *Citrullus* cultivars.

In most studies (Pofu and Mashela, 2011; Pofu et al., 2011b; Pofu et al., 2010a, b), most second-stage juveniles of *M*. species were converted into males, due to their failure to establish feeding sites in *C*. species (Ferraz and Brown, 2002). The observation of conversion of juveniles to males confirmed other observations in other plant species with resistance to *M.* species (Fassuliotis, 1970). The biological importance of conversion of nematode juveniles into males is that the latter do not feed and are also not required for reproduction (Ferraz and Brown, 2002).

INTER-GENERIC GRAFTING TECHNOLOGY

Grafting technology in vegetable production for managing soil-borne diseases is gaining popularity, particularly in countries with stringent environmental laws (Traka-Mavrona et al., 2000; Tsambanakis, 1984). Inter-specific grafting is common with a particular genus, which contains genotypes with resistance to soil-borne pathogens (Pofu and Mashela, 2011b). In the genus *Citrullus*, for instance, there are no genotypes, which are resistant to *M*. species (Thies and Levi, 2006; 2007). Inter-generic grafting has had incompatibility challenges due to different stem diameter sizes of scions and rootstocks in different genera (Tiedermann, 1989). Watermelon–with relatively thick stem diameters when grafted onto *C. africanus* and *C. myriocarpus*, relatively thin stem diameters, resulted to less than 36% survival (Pofu and Mashela, 2011a). Through intensive research and development, appropriate procedures were developed to optimise the stem diameters of the two genera, resulting into 100% survival of inter-generic grafts (Pofu and Mashela, 2011a). In a subsequent greenhouse trial, (Pofu et al., 2011b), all inter-generically grafted seedlings also survived with *C. africanus* and *C. myriocarpus* seedling rootstocks retaining their capabilities to suppress population levels of *M. incognita* race 2. Under field conditions, the procedure was also successful, with inter-grafts flowering earlier and producing higher fruit yield than intact plants (Pofu et al., 2011a).

CONCLUSIONS

Indigenous *Cucumis* technologies, comprising of four different techniques, had been successfully researched and developed for managing plant-parasitic nematodes, particularly the root-knot nematodes, in tomato production in low-input agricultural farming systems. The technologies have the potential for expanding to other farming systems and plant-parasitic nematodes. For instance, fermented crude extracts have the potential for use through "botinemagation" systems in large-scale commercial irrigated agriculture, whereas *Citrullus-cucumis* grafts have the potential for use in commercial watermelon production systems, all for managing nematode levels as alternative to synthetic nematicides and/or conventional organic amendments.

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