Assessing the potential of kava (*Piper methysticum* Forst) and wild kava (*Piper aduncum* L.) as organic amendments for managing root-knot nematodes

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ABSTRACT
*Kava (*Piper methysticum *Forst) and wild kava (*Piper aduncum *L.) were evaluated for their efficacy against root-knot nematodes. Plant materials were tested as soil additive in pot trials at 2% and 4% concentrations for 0, 1 and 2 week degradation periods. Effects on the root-knot nematode, M. incognita, and its host, tomato, were recorded. All the tested materials reduced the number of galls compared to the control. Generally, maximum gall suppression was achieved at higher concentration (4%) and with no degradation time. Gall suppression was directly related to concentrations of plant materials, but not with the degradation time after soil incorporation. Kava powder, kava peelings and kava kosa caused maximum gall suppression but were phytotoxic. Kava stem, kava leaf and wild kava leaf, on the other hand, resulted in lower gall suppression but enhanced plant growth. These plant species are good candidates for further trials as soil amendments.*
Key Words: *Kava,* Piper aduncum*,* Piper methysticum*,* Meloidogyne incognita*, soil amendment, tomato.*

1 INTRODUCTION

Root-knot nematodes, Meloidogyne spp., are sedentary endoparasites capable of infecting over 3000 plant species (Shurtleff and Averre, 2000; Abad et al., 2003). Root-knot nematodes are serious pests of agricultural crops. Management of Meloidogyne spp. and other plant-parasitic nematodes has been challenging worldwide, particularly due to reduced availability or complete ban of effective chemical nematicides such as methyl bromide (Braga et al., 2001; Hooks et al., 2006). Major limitations to wide use of synthetic nematicides, include their hazards to environment, toxicity to important non-target organisms including humans, high cost, and supply limitations in developing countries (Parmar and Walia, 2001). This has prompted the search for environmentally benign and safe alternative control methods. As the search for alternative strategies intensifies, the use of plant-based organic amendments is rapidly gaining importance.

The use of organic amendments alone or as part of an integrated pest management program is a promising alternative for managing root-knot nematodes. Plant materials used as organic amendments generally release antagonistic or nematotoxic compounds such as phenols, ammonia, nitrates and volatile fatty acids upon degradation (Ritzinger and McSorley, 1998; Ritzinger et al., 1998). Organic amendments also provide additional nutrients for plants and improve soil properties (Maloy, 1993; Crow et al., 1996; Braga et al., 2001). The efficacy of an organic amendment against nematodes would depend on the presence and concentration of nematotoxic compounds in the amendment, their degradation properties, the amount of amendment used and the effect of the amendment on crop health. Relatively rapid biodegradation of most organic amendments results in short-term effectiveness, therefore, higher dosage or frequent application is required to achieve similar levels of efficacy similar to those of synthetic nematicides (Isman, 2001). The concentration of the compounds and (or) the amendment, and the time required for the degradation of the amendment are two important parameters related to the efficacy of plant materials against nematodes.

Organic amendments obtained from various plant families such as *Asteraceae, Fabaceae, Meliaceae* and *Euphorbiaceae* have shown varying degree of success in controlling plant parasitic nematodes (Ritzinger and McSorley, 1998; McSorely *et al*., 1999; Pathak *et al*., 2001; Morris and Walker, 2002; Wang *et al*., 2002; Zasada *et al*., 2002; Dijan-Caporalino *et al*., 2005). Plants from the family *Piperacae* have been traditionally known for their medicinal and insecticidal use as well as for spices (Arnason *et al*., 2005). In the Pacific region, including Fiji, *Piper methysticum* Forst (Kava) from the family *Piperacae* is widely grown and consumed as a traditional drink. Kava has a wide range of properties including pharmacological, antimicrobial, antifungal and medicinal applications (Shulgin, 1973; Duve, 1976; Wu *et al*., 2002; Cote *et al*., 2004). Kavalactones are the most important biologically active chemical compounds found in kava (Coulter *et al*., 2007). Khurma and Singh (2005) reported nematicidal effects of kava powder on root-knot nematodes. In addition to nematicidal activity, kava powder has been reported to possess herbicidal activity against barnyard weed (Xuan *et al*., 2003; Xuan *et al*., 2005).

Kava contains several known biologically active compounds including desmethoxyyagonin, kavain, 7,8-dihydrokavain, yagonin, methysticin, and dihydromethysticin which have herbicidal and antifungal activities (Xuan *et al.,* 2006). Wild kava (*Piper aduncum* L.) also belongs to the family *Piperacae* and is closely related to kava. Wild kava is known to contain compounds such as dillapiol and benzoic acid derivatives, many of which exhibit antimicrobial, molluscicidal, fungicidal, germination inhibition and insecticidal activity (Baldoqui *et al*., 1999; Arnason *et al*., 2005; Navickiene *et al*., 2006). There is no report on the nematicidal properties of wild kava.

The objective of this study was to assess the efficacy of kava and wild kava against root-knot nematodes, their effect on host plant health, and to determine the effective

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concentrations and degradation times for nematicidal activity.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS

Kava powder and kava peelings which are commercially available in markets throughout Fiji were purchased from the Nausori market, Fiji. The kava powder was directly sieved while kava peelings were first blended and then sieved (2mm pore size) to remove coarse particles. Kava kosa, a waste material left after squeezing and straining kava powder with water in cheesecloth while preparing the kava beverage for consumption, was collected from household kava consumers and air-dried to remove moisture. The dried kava kosa was also sieved through a 2mm sieve. Fresh kava stems and leaves (cv. Kasa Loa) were obtained from the Koronivia agriculture research station plant nursery located in Nanduruloulou, Fiji. The fresh stems and leaves were cut into small pieces and then macerated separately in a blender to form a homogenous paste. Wild kava *(Piper aduncum* L.*)* leaves were collected from the University of the South Pacific botanical garden and prepared as for the leaf and stem tissue described above.

2.2 NEMATODE INOCULUM

Bean (*Phaseolus vulgaris*) plants infected with *Meloidogyne incognita* (Kofoid & White) Chitwood were collected from a vegetable garden in Suva, Fiji. The egg masses were separated from infected bean roots and placed in distilled water to hatch in a BOD incubator set at a temperature of 28 $\pm$ 1^{o} C. Second stage juveniles (J2) were used as inoculum for the experiment.

2.3 HOST PLANT

Tomato plants (*Solanum lycopersicum* cv. Moneymaker) were used as the host plant for the experiment. Seedlings were grown in autoclaved soil and transplanted once they were four weeks old.

2.4 EXPERIMENTAL SETUP

Kava plant materials were weighed at 2% (8g) and 4% (16g) concentrations (v/v) and placed in separate labeled plastic bags where they were thoroughly mixed with 400g of autoclaved soil. The amended soil was then transferred to plastic pots of diameter 3 inches and height 4 inches which were placed in a glasshouse for 0, 1, or 2 weeks prior to transplanting tomatoes to allow different degradation times for the plant material. Cotton wool was placed at the bottom of each pot to prevent the loss of amended soil. Due to limited availability, kava leaf material was tested only for the 2-week degradation period. Control pots received no amendments. All pots were placed in a random block design in the glasshouse and each treatment had four replicates. *Meloidogyne incognita* J2 were collected for 3-5 days and a suspension in distilled water was prepared. Each plant was inoculated by pipetting 1 ml (100 J2) into each of two wells made near the base of the plant in each pot. Inoculation was done 1 day after transplanting the seedlings. The plants were kept in the glasshouse for five weeks after transplanting and watered as required.

2.5 DATA ANALYSIS

The experiment was terminated at the end of fifth week after transplanting. The plants were removed from the pots and gently washed free of soil before being taken to the lab for recording observations. Root galls and egg masses were counted by examining the roots under stereoscopic microscope. The percentage of the total root system galled was determined (Sikora & Fernandez, 2005), and plant height, weight, number of leaves, branches and development of root system were recorded as quantitative parameters indicating plant health. The data is summarized in Table 1.

The data was statistically analyzed using SPSS software version 13.0 to carry out univariate analysis of variance, Pearson correlation, and to determine the mean and standard error. Figures 2 and 3 represent the analyzed data.

3 RESULTS

The root galling ranged from 0 to 35% of the root system. The maximum root galling was found in the unamended controls and the minimum with kava powder and peelings (Table 1). For all the materials, the greatest gall suppression was achieved where no degradation period was given, but, with the exception of 4% wild kava leaves, which improved the plant health and suppressed the galls to some extent. Transplanting immediately after incorporation of all other materials resulted in suppressed plant growth.

Kava powder and kava peelings were the most effective in suppressing the root galls at both concentrations and all three degradation times. However, both kava powder and kava peelings negatively affected the growth of plants, and in some cases transplant mortality. The most severe effect on plant health was observed at higher concentration and lesser degradation time with 75% transplant mortality for 4% kava powder with 0 and 1 week degradation and 50% mortality with 2 weeks degradation. Transplant mortality was also recorded in case of 4% kava peelings with both 0 and 2 weeks degradation. There were no transplant deaths at 2% concentration of kava powder and peelings but plant growth was suppressed as compared to the control. The healthy transplants used to replace the dead plants (not included in statistical analysis), did not have any galls, and still had poor plant growth.

The concentrations of kava powder and peelings did not have a significant difference in effect ($P \geq 0.05$) on the number of galls, probably because even 2 % dose achieved nearly maximum possible gall suppression. All the other amendments had a direct relationship between the concentration and the number of galls present.

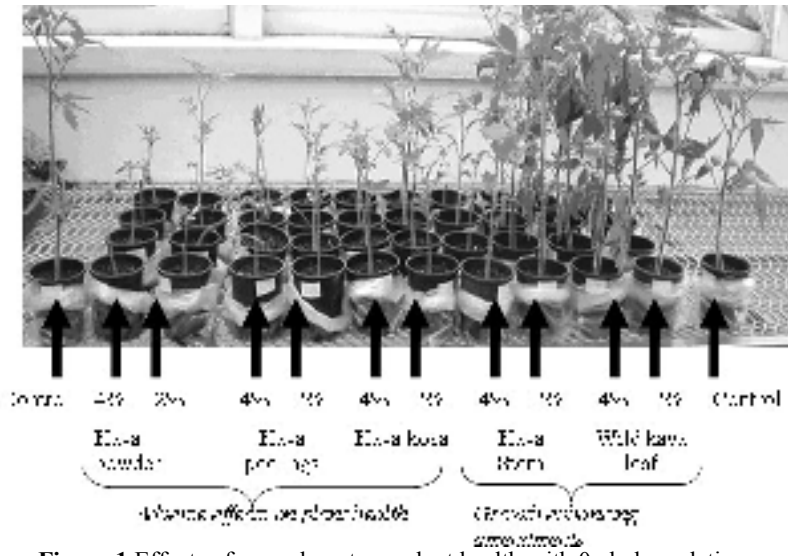

Figure 1 Effects of amendments on plant health with 0wk degradation
(Note: Similar effects were observed for amendments with 1 and 2 weeks degradation)

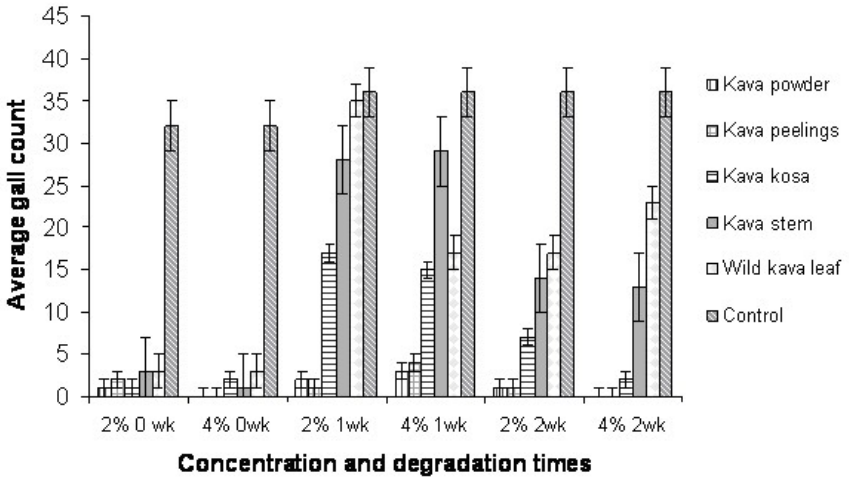

Figure 2 Effect of organic amendments on gall count

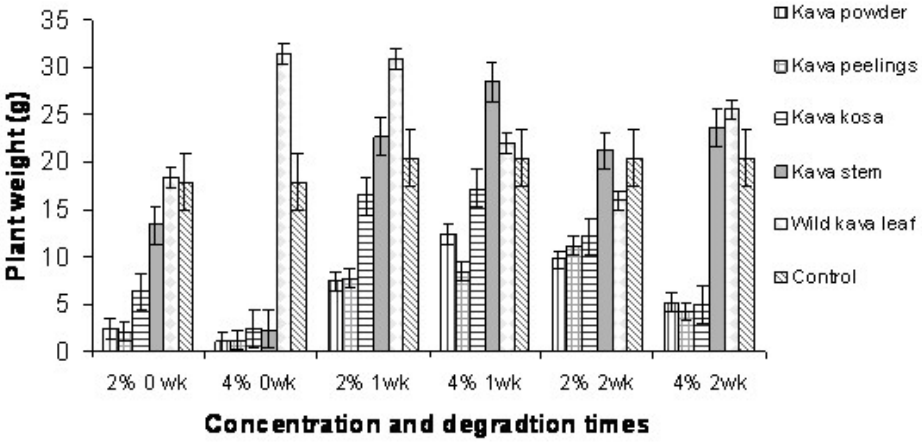

Figure 3 Effect of organic amendments on plant weights

Table 1 Effects of amendments on gall count and tomato plant parameters

Plant parameters	Kava Powder		Kava Peelings		Kava Kosa		Kava Stem		Kava Leaf		Wild Leaf	Kava	Control
Average Gall Count	**2%**	**4%**	**2%**	**4%**	**2%**	**4%**	**2%**	**4%**	**2%**	**4%**	**2%**	**4%**	
0 wk	1	0***	2	0 **	1	2	3	1			3	3	32
1 wk	2	3***	1	4	17	15	28	29			35	17	32
2wk	1	0**	1	0 **	7	2	14	13	14	8	17	23	36
Plant Weight [g]													
0 wk	2.4	1.0	2.1	1.2	6.3	2.4	13.3	2.3			18.4	31.4	20.5
1 wk	7.4	12.3	7.7	8.4	16.4	17.2	22.7	28.5			30.9	22.0	20.5
2 wk	9.7	5.1	11.2	4.2	12.1	4.9	21.2	23.6	22.7	19.2	15.9	25.6	17.9
Plant height [cm] 0 wk	17.3	14.2	16.3	12.9	26.0	15.5	34.5	17.4			33.8	39.8	34.4
1 wk	27.0	32.6	24.6	27.7	40.0	37.6	41.6	41.5			40.7	39.9	34.4
2 wk	31.5	21.9	34.6	19.7	39.5	25.6	41.0	45.0	42.3	37.5	40.2	41.0	41.2
Number of leaves 0 wk	17	9	19	14	35	18	45	23			42	61	52
1 wk	38	41	35	38	55	53	66	70			87	67	52
2 wk	36	32	47	27	52	34	68	71	68	62	62	69	61
Branching													
0 wk	4	4	5	4	7	4	9	6			8	9	8
1 wk	7	7	7	7	8	7	9	10			12	9	8
2 wk	7	7	8	6	9	7	10	10	10	9	10	10	9
Root system*													
0 wk	4	4	4	4	3	4	3	4			2	1	2
1 wk	3	4	3	4	2	3	1	1			1	2	2
2 wk	4	4	3	4	2	3	1	1	1	2	1	1	2

KEY

*** indicates severe phytotoxic effects i.e. 75% transplants died out before termination.

** indicates phytotoxic effects i.e. 50% transplants died out before termination.

* represents root system development rated using scale of 1, 2, 3, 4

1= Well developed root system with extensive secondary root system branching from the main root.

2= Root system well developed but has less extensive secondary root system branching from the main root.

3= Poorly developed root system with very little secondary branching from the main root.

4= Very poorly developed root system with few short secondary root.

Note: The table contains additional plant parameters measured plus data for Kava leaf treatment which are not included in Figures 2 and 3.

There was significant ($P \leq 0.05$) interaction between concentration*degradation time and amendment* degradation time on the plant weight and number of galls respectively hence the degradation time of all the amendments has an effect on both the number of galls present and the plant health as shown in Figures 2 and 3.

The degree of gall suppression by kava kosa for both concentrations with no degradation period was similar to that of kava powder and peelings. Kava kosa caused relatively less gall suppression and adverse effect on the tomato plants than kava powder or peelings at both concentrations, and with either 1 or 2 weeks degradation period prior to transplanting.

Transplanting immediately after incorporation of 2 % or 4% kava stem was effective in suppressing galls but the tomato plant growth was poor. Gall suppression decreased with 1 and 2 week degradation periods, and plant growth was enhanced. Kava stem was comparatively less effective after 1 and 2 week degradation when compared to kava powder, peelings and kosa.

Kava leaf was used as an amendment only for 2 weeks degradation period. The degree of gall suppression with kava leaf was higher at 4% than at 2% dosage, but overall kava leaves were less effective than kava kosa, peelings and powder. Kava leaf at both concentrations improved plant growth.

Wild kava leaf had some gall suppressive ability where transplanting was done immediately after amendments were applied. The effectiveness of wild kava leaf decreased substantially with degradation time with very little gall suppression achieved with either 1 or 2 weeks degradation periods (Figure 2). At 4%, wild kava enhanced plant growth considerably in all treatments.

4 DISCUSSION

The strongest effect against the nematodes was noted for both kava powder and peelings. Their phytotoxicity to tomato makes them relatively unattractive as organic amendments for nematode suppression, but they could be potential sources of nematicidal compounds. The

breakdown products after a 2 week degradation period have greater nematotoxicity than from 1 week degradation, a trend that was also observed for the most other tested amendments (Figure 2). In the pots where transplants were killed, new transplants used to replace the dead plants for observational purposes had no galls and showed poor growth, indicating the persistence of toxic effects of the amended soil. The phytotoxic and nematotoxic effects of kava powder on tomato were also observed by Khurma and Singh (2005). Lower phytotoxicity of kosa may have been due to the removal of some of the active compounds during the mixing, squeezing and straining of kava powder with water while preparing kava beverage. The fact that kava kosa, which is a waste material, was active enough to suppress the RKN makes it a good candidate for further trials.

Kava contains a number of bioactive compounds such as kavalactones and phenols, with most of the bioactivity attributed to kavalactones (Coulter *et al*., 2007). Kavalactones such as desmethoxyyagonin, kavain, 7,8-dihydrokavain, yagonin, methysticin, and dihydromethysticin have been shown to possess herbicidal and antifungal activities (Xuan *et al.,* 2006). Kava powder, upon degradation, also releases a range of phenolic compounds that were considered responsible for inhibiting barnyard weed germination and growth for 25 days after application (Xuan *et al*., 2003; Xuan *et al*., 2005). Phenolic compounds released from a range of other organic amendments are also known to have nematicidal properties (Dijan-Cporalino *et al*., 2006; Ritzinger *et al*., 1998; Ritzinger and McSorley, 1998). The phenolic compounds, therefore, may have caused nematotoxicity after 2 weeks degradation as well as contributed to some phytotoxicity in the current investigation

Kava stem contains compounds similar to those in kava powder but in lower concentrations (Coulter *et al*., 2007). Both kava stem and kava leaf are able to suppress galling as well as to enhance plant growth, so both have the potential to be used as green manure. Unfortunately, their cost and limited availability does not favor their use as routine organic amendments. Interestingly, wild kava leaf improved plant growth substantially even though it had low gall suppression. Wild kava leaf is readily available at relatively low cost, so could be a cost effective amendment in combination with other control methods. Wild kava leaves are known to contain phenolic compounds such as phenylpropanoids, benzoic acid derivatives, chromenes and flavonoids (Baldoqui *et al*., 1999; Navickiene *et al*., 2006) which may be nematotoxic but the compounds in wild kava leaves responsible for improved plant health are yet to be determined.

All the variables (organic amendment, concentration and degradation time) tested had an effect on both the number of galls and plant health (Figures 2 & 3). The number of galls was positively correlated to plant health parameters based on Pearson correlation test ($p<0.05$). This is puzzling since normally the gall numbers are negatively correlated to plant health (Manzanilla-Lopez *et al.*, 2004) but due to sufficiently low galling severity (about 35%) the effect of amendment on the plant health masked the effect of the nematode.

An ideal organic amendment should be able to suppress nematodes, allow a crop to be planted after a reasonable degradation period after incorporation, and remain effective over time. Kava powder, peelings and kosa need to be tested at degradation periods longer than two weeks to determine the period that nematotoxicity persists with minimum or no phytotoxicity. Using these materials in combination with growth enhancing amendments or other products to determine whether their phytotoxicity can be buffered is another option. For instance, wild kava leaf that is able to improve plant growth but has lower gall suppression could be tested in combination with a more nematotoxic material such as kava kosa at different concentrations and degradation times. The ready availability and cost effectiveness of kava kosa and wild kava leaf is an important factor and an advantage over the other materials that paves the way for further studies. Further trials are being undertaken to support these findings and to have more conclusive observations.

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