International Journal of Science, Technology and Society
2015; 3(3): 76-82
Published online April 15, 2015 (http://www.sciencepublishinggroup.com/j/ijsts)
doi: 10.11648/j.ijsts.20150303.12
ISSN: 2330-7412 (Print); ISSN: 2330-7420 (Online)

Effect of Diatomaceous Earths on Mortality, Progeny and Weight Loss Caused by Three Primary Pests of Maize and Wheat in Kenya

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To cite this article:
Ngatia Christopher Mugo, Mbugua John Nderi, Mutambuki Kimondo. Effect of Diatomaceous Earths on Mortality, Progeny and Weight Loss Caused by Three Primary Pests of Maize and Wheat in Kenya. *International Journal of Science, Technology and Society.* Vol. 3, No. 3, 2015, pp. 76-82. doi: 10.11648/j.ijsts.20150303.12

Abstract: The Kensil fine (KF) dust was evaluated under laboratory conditions for the control of three important storage insect pests of maize and wheat. Serial concentrations of KF, Dryacide (DA) and Wood ash (Ash) were admixed with 100g of maize or wheat in ventilated glass jars. Mortality of *S. zeamais, P. truncatus* and *R. dominica* was assessed at 7, 14, 28, 56 and 84 days interval after grain treatment. At 28 days, all the three dusts effectively controlled *S. zeamais* with 95% - 100% mortality while only DA was effective against *P. truncatus*. Both KF and Ash, with 84% and 92% mortality, did not reach the threshold required for *P. truncatus.* Mortality in *R. dominica* only peaked after 56 days but again only DA treatment was effective at 84 days. The delayed effect of the Diatomaceous earths (DE) and ash treatments appear to contribute to the higher damage inflicted; hence more weight loss than was expected. At 28 days mean sample weight loss by *S. zeamais* was 4.5% while *P. truncatus* and *R. dominica* caused 4.2% and 3.5% respectively. The emerged progeny after 14 days exposure to the three dusts was different for each pest with DA producing the least and KF the most. These results formed the criteria on which to base future trials under simulated farmer storage practice.

Keywords: Diatomaceous Earths, Delayed Effect, Grain Storage, Farmer Practice, Pest Control

1. Introduction

Storage insect pests have been linked with reduced income and food insecurity at farm household level (Stathers, 2002). Studies show the *Sitophilus zeamais* Motschulsky and the *Prostephanus truncatus* Horn are among the main storage insect pests of maize, (Golob et al., 1996; Brice et al., 1996, Marshland & Golob 1996; Donaldson et al., 1996) while the *Rhizopertha dominica* prefers smaller grains like sorghum and wheat (Navarro and Donahaye, 1976). In their effort to mitigate losses, farmers either sell their harvested grain early or use different means of grain protection including traditional methods (Golob, et al., 1983). The use of chemicals among farmers has been on the rise, though the impact of insect infestation in terms of grain damage and weight loss also appears to display an upward trend (de Lima 1979; Muhihu and Kibata, 1985; Mutambuki and Ngatia, 2006).

Coupled with inherent problems like the development of resistance, risks of exposure to toxic pesticides, environmental contamination and zero tolerance in the grain trade, the urge to replace chemical pesticides with effective, safe and environmentally friendly pest control products seems imminent. Kuronic, (1997) noted that diatomaceous earths (DEs), the fossilized skeletons of diatoms, have the greatest potential to replace pesticides. They have low mammalian toxicity (Golob 1997 and Kuronic 1998) and can control a wide range of stored products insect pests (Barbosa et al., 1994; Subramanyam et al., 1998; Mewis & Ulrichs 2001; Arthur, 2002; Arthur & Throne 2003; Athanassiou et al., 2005; Wakil et al., 2010). Their potency does not expire and can therefore protect grain during a storage season (Stathers, et al., 2004). Ebeling (1971) explains that DEs work by absorbing the epicuticular lipids which leads to excessive water loss and death of insects.

DEs are found in different parts of the world, and based on their physical properties and the diatom species, Kuronic (1997, 1998) found significant differences in their efficacy against insects. Athanassiou, et al., (2003) added the grain type (Fields & Kuronic 2002; Nikpay 2006; Athanassiou et al., 2006) and grain moisture, temperatures and relative humidity also influence their efficacy. The fact that DEs can combine with chemical pesticides, (Stathers, 2002; Ceruti and Lazzari, 2005), or bio-control organisms to enhance potency (Lord, 2001; Akbar et al., 2004) appear to attract local commercial interests aimed at broadening the areas of use for the DEs. The main hindrance is the stringent regulatory requirements enforced by the Pest Control Products Board (PCPB) which recommends local efficacy trials before any pest control product can be registered. Towards this requirement, the African Diatomite Industries Limited (ADIL), requested the Kenya Agricultural Research Institute (KARI) to evaluate Kensil F before seeking its registration for use in the storage sector. This paper describes the laboratory evaluation process for Kensil Fine (KF) dust which was compared with Dryacide (DA) from Australia and Wood ash (Ash) for their control of three important stored products insect pests.

2. Materials and Methods

2.1. Grain Conditioning and Treatment

A 90kg bag of freshly harvested maize and 45 kg of wheat were fumigated in metal drums using phosphine gas generating tablets for 7 days. The grain was then screened over a sack sieve (maize) and 1mm aperture test sieve (wheat) to remove dust and non-grain material. A 100g of maize were put in each of the 720 glass jars of 300cc capacity, half of which were closed with wire gauze and the remaining with watman filter papers. The jars closed with wire gauze were then grouped into 4 lots of 90 and again divided into 5 batches of 18 jars of which a set of 3 replicates was treated with 0, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%w/w serial doses of Kensil F, Dryacide or Wood Ash. The same was repeated for the jars closed with filter paper. A similar quantity (100g) of wheat in another 360 jars closed with filter paper was treated in the same way.

2.2. Introduction of Test Insects

Sitophilus zeamais (Motschulsky) *Prostephanus truncatus* (Horn) and *Rhizopertha dominica* (F.), all ex laboratory cultures maintained on whole maize and wheat grain at 25±5°C and 70%±2% relative humidity (r.h) were used. Twenty unsexed but active adult *S. zeamais* were introduced into each of the 90 jars with maize treated with KF and repeated for jars treated with DA and Ash respectively. A similar number of *P. truncatus* adults were introduced into the maize jars closed with wire gauze (to check escape) and treated with the three dusts. Finally, the process was repeated for *R. dominica* in wheat jars. All the jars were randomly placed in the temperature control room (TCR) set at 25±5°C and 70±2% relative humidity. Mortality of the parent population was assessed after 7 days exposure for each of the post-treatment intervals of 7, 14, 28, 56 and 84 days. Any increase especially after 28 days was classified as the F_1 progeny.

2.3. Progeny Monitoring

Progeny monitoring was done only in the jars exposed for 14 days. After accounting for the parental population, the jars were incubated in the same CTR for a further 21days before the contents were sieved and the status of the recovered adult insects noted. Sieving to remove emerged insects was repeated at 2-day interval until all the jars failed to produce any adults for three consecutive attempts when the total emergence per jar was noted.

2.4. Estimate of Weight Loss in Samples

Grain weight reduction occasioned by insect feeding was calculated from the differences between the original and the final weight in each jar and the results expressed as percentage using Harris and Lindblad (1978) derivative formula below:

$$\%\text{wt loss} = \frac{w_1 - w_2 \times 100}{w_1}$$

Where
W_1 = Original weight without inert dust;
w_2 = Final weight without inert dust.

2.5. Data Handling and Analysis

Data on mortality, F_1 emergence and weight loss was managed with the Excel and analyzed using the statgraphic softwares. ANOVA indicated the main factors that influenced insect mortality while Least Significant Difference (LSD) separated treatment means that significantly contributed to the difference at 95% level of confidence.

3. Results and Discussion

3.1. Results

3.1.1. Mortality of Test Insects Exposed to DE on Treated Maize or Wheat

The ANOVA showed that post-treatment period, pest species and applied dust treatments significantly (P=0.0000) influenced mortality of test insects (Table 1). Insect mortality was different from one interval to the next and for *S. zeamais* and *R. dominica* it increased with storage period. Mortality of *P. truncatus* fluctuated between intervals and Dryacide was the most effective among the three dusts.

Table 2 shows the effect of the three dusts on the mortality of the test insects. At 7 days, Kensil F controlled only 37% of the *S. zeamais,* which increased to 97% at 28 days. Dryacide and Ash were significantly (P =0.0003) better at 69%, rising to 100% and 96% respectively for same period. After 28 days, insect mortality dropped but increased again at 84 days.

Mortality in *P. truncatus* was much lower, at between 23% and 44% at 7 days across dust treatments and thereafter fluctuated up and down between intervals. Again, Kensil F performed poorly and was not significantly (P=0.05) different from Ash. Between 7 and 14 days, mortality in Dryacide treatment was significantly different from the control. At 28 days, the three dusts controlled 84%, 96% and 92% respectively showing great improvement. After 28 days, pest mortality declined but Dryacide maintained a clear lead from the other dusts.

When applied on wheat to control *R. dominica,* the three dusts recorded even lower mortality compared with that in *S. zeamais* and *P. truncatus* in maize. At 7 days, Kensil only achieved 11% compared with 17% and 25% for Ash and Dryacide respectively. At 28 days, mortality in Kensil treated maize had risen almost by four-folds to 43% while that in Dryacide treatment had increased by more than three-folds to 92%. Dryacide then progressed to effective level (>95%) at 84 days while both Kensil F and Ash only attained 68% and 81% respectively at 56 days. The data portrays a close relationship between Ash and Kensil and that the three dusts were a better alternative to no control. Figure 1 shows the performance of individual dusts based on average mortality as compared with the control. Among the dusts the superiority of Dryacide as a grain protectant was confirmed. *S.* zeamais was the most susceptible followed by *R. dominica* and *P. truncatus.* All the dust treatments indicate the benefits to be gained if farmers could use them for protecting stored grain.

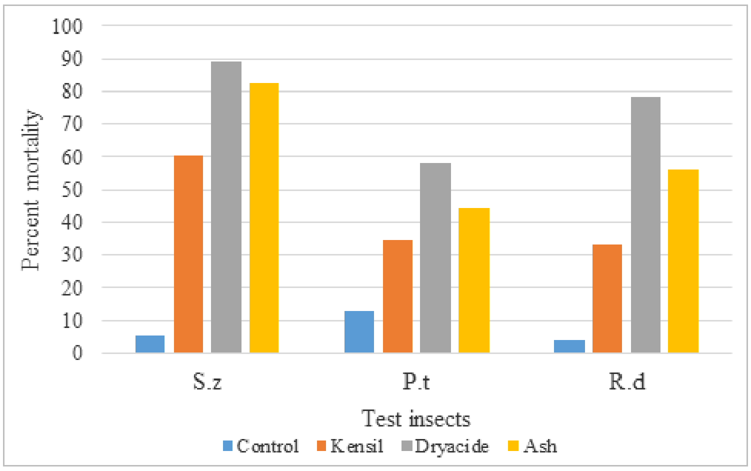

Fig. 1. *The influence of dust treatments on the mortality of three test insects as compared with the control. Key: S.z = Sitophilus zeamais; P.t = Prostephanus truncatus; R.d = Rhizopertha dominica.*

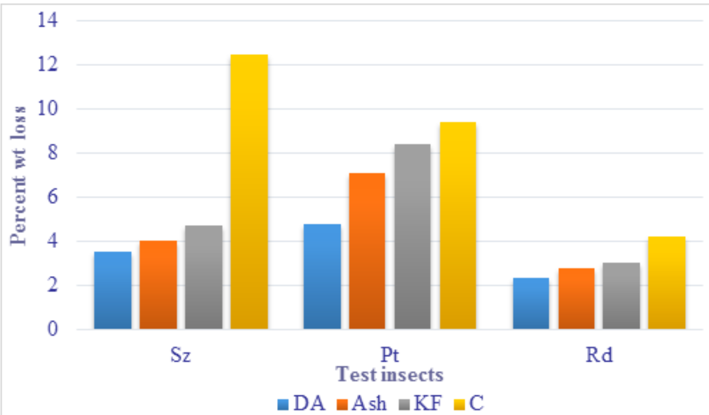

Fig. 2. *The influence of dust treatments on percent weight loss in grain samples exposed to three test insects. Key: S.z = Sitophilus zeamais; P.t = Prostephanus truncatus; R.d = Rhizopertha Dominica; DA = Dryacide, KF = Kensil fine, C = control.*

3.1.2. The Potential of DE to Protect Stored Grain from Damage by Insect Pests

The potential of Kensil Fine to protect stored grain was assessed from the level of grain damage and its subsequent weight loss. Table 3 shows the level of sample weight loss in both maize and wheat when compared with the control. At 14 day interval, dust treatments recorded between 2% and 3% in *S. zeamais* infested maize compared with almost 7% for the control. Dryacide had the lowest while there was no statistical difference between Ash and Kensil. At 28 days, both Ash and Kensil recorded 4.6% and 5.1% weight loss, an indication that their effectiveness was weak, a situation which persisted to 84 days. With 2.1% - 4.4% weight loss, Dryacide was the only effective protectant against *S. zeamais*. The benefit of applying protection can be worked out from comparing treatment figures against the control at each interval. *P. truncatus* appear to be the most damaging, and despite the dust application, weight loss between 4.6% and 10.3% was recorded at 7 days across the treatments. However, weight loss dropped by a factor of between 0.4 and 0.8 (20%

and 59%) at 28 days, but gradually increased to 6.5% - 13.7% at 84 days. At every interval, Dryacide had the lowest figures and compared with Kensil, the differences were highly significant (P=0.0000), only comparable with the control. Wheat suffered between 2% and 6% weight loss from *R. dominica* infestation in spite of the treatments applied. At 14 days, all treatments recorded between 1.9% and 2.3% weight loss as compared with 3.1% for the control. Highest weight loss (of between 3.1% and 3.8%) was recorded at 28 days with Dryacide having the lowest and Kensil the opposite. From 28 days, sample weight loss progressively dropped and at 84 days only Kensil reached 3.3% level. However, all treatments were significantly (P <0.05) better than the control. Figure 2 illustrates the protective benefit accorded by the applied dusts on stored grain against the damage from test insects. It is clear that more benefit would be realized if the pest was *S. zeamais* and not others.

3.1.3. Emergence of F1 Progeny of Test Insects from DE Treated Grain

Varying numbers of F_1 progeny emerged from the eighteen jars that were used from each treatment (Table 4). More progeny emerged from maize than wheat and *S. zeamais* had consistently lower numbers (between 7 and 30), compared with 19 – 87 for *P. truncatus.* It was not clear why *R. dominica* produced negligible progeny of between 1 and 2 adults across the treatments including the control. Figure 3 shows the influence of the dust treatments on the emerged progeny. Although all treatments significantly (P=0.0000) suppressed progeny emergence, Dryacide was markedly better with below 20 adults. Kensil and Ash could not effectively suppress *P. truncatus,* the most destructive of the three test insects, indicating reduced protection of grain.

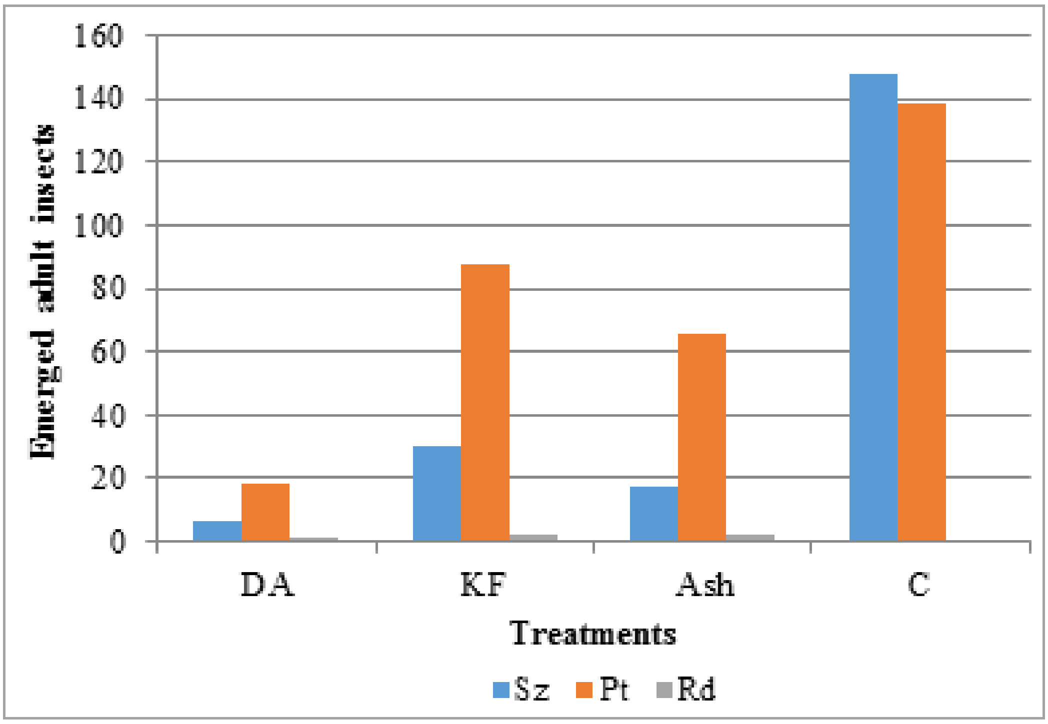

Fig. 3. *Progeny that emerged from Sitophilus zeamais (Sz), Prostephanus truncatus (Pt) and Rhizopertha dominica (Rd) after 14 day exposure on maize/wheat treated with Dryacide (DA), Kensil fine (KF) and Ash compared with untreated control.*

3.2. Discussion

The lab bioassay was set up to generate various data sets that could give a trend in efficacy of Kensil F, a locally mined diatomaceous earth. From the results, the mode of action clearly emerged where mortality was gradual, reaching the peak in 28 days for *S. zeamais* and *P. truncates* respectively. The peak for *R. dominica* was at 56 days. This contrasts with findings in some lab studies where DEs recorded 100% mortality between 7 and 14 days (Collins and Cook, 2006; Athanassiou et al., 2004). In this study the delay was probably due to the absorptive process of the epicuticle described by Ebeling (1971), a clear difference from the almost instant mortality normally observed in chemical trials. Although mortality trend was similar for all test insects, the level of control showed different pest responses with *Sitophilus zeamais* being more susceptible than the grain borers. Kuronic (1997 and 1998) had stated pest species as a factor in DE efficacy, which could partly be due to specific pest behaviour (borers, in the open tend to be docile and less active while they actively bore into the grain almost immediately after introduction). Such behaviour tend to reduce the chances of abrasion from the dusts which would improve if the insect moved about among DE treated grain. With delayed mortality, test insects had ample time to bore into grain, feed and reproduce, a fact confirmed by a second grain damage peak at 12 weeks. The impact of insect damage directly relates to percent weight loss noted. Grain damage is therefore the main criterion used to judge the protection capacity of a candidate product. The Pest Control Products Board, a Kenya Government regulatory body considers >5% as the ceiling for acceptance to license and register chemical pesticides for use in the storage sector. Higher damage levels also translate to higher weight loss which has a bearing on market value and trade. In this trial, the extensive damage by *P. truncatus* was thus responsible for higher (>5%) weight loss especially in maize treated with either Ash or Kensil F. This again was a reflection of the pest behaviour, and between the two borers, *P. truncatus* was the more voracious feeder and quickly bored into the grain thereby avoiding too much contact with grain protectant. These assumptions will be considered during the simulation trial. The data also allows the calculation of differential loss farmers would suffer by applying a less effective dust. Using Dryacide as the best option during this trial, a farmer would suffer 1.21% for applying Kensil F on maize infested with *S. zeamais*, or 3.6% if *P. truncatus* was the pest. For wheat, the difference was much smaller (0.35%) but the proportional loss could be higher considering the grain size compared to that of maize.

4. Conclusion

The rising interest in DEs has been responsible for many studies some of which have led to commercial exploits. This pilot trial will contribute greatly to the understanding of how the DEs work before formal registration for use in the storage sector in Kenya. It will also help to expand the areas of use for Kensil, while farmers will have a novel alternative to chemical pesticides. Though DEs have a definite role to play in the management of storage insect pests, the study has shown that they are not instant in their efficacy as would be for chemical pesticides. They require actively mobile insects among the treated grain to abrade the waxy layer which prevents excessive water loss from their bodies. These were the behavioral differences between *S. zeamais* and *P. truncatus*. The data generated promising trends and it was left to the proprietor to consider refining the physical properties (size) of Kensil before the research body could plan advanced trials.

Table 1. *ANOVA for mortality of test insects exposed to Kensil F, Dryacide and Ash*

Source	Sum of Squares	df	Mean Square	F- Ratio	P-Value
MAIN EFFECTS					
A: Weeks	171997	4	42999.2	82.79	0.0000
B: Pests	113318	2	56658.9	109.09	0.0000
C: Treatment	233782	3	77927.0	150.04	0.0000
D: Reps	640.631	2	320.316	0.62	0.5400
RESIDUAL	367721	708	519.38		
TOTAL (CORRECTED)	88745	719			

All F – ratios are based on the residual mean square error.

Table 2. *Percent mortality of test insects when exposed to three inert dusts applied on stored maize or wheat*

Maize weevil (Sitophilus zeamais)						
Treatments Post treatment period (weeks)						
1	2	4	8	12	Mean	
C	8.3a	11.7a	1.7a	2.3a	3.7a	5.54
KF	37.3a	52.0b	97.0b	38.3b	77.6b	60.44
DA	68.7b	94.7c	100b	83.1d	98.6c	89.02
Ash	68.9b	82.7c	95.7b	72.1c	92.9c	82.46
P value	0.0003	0.0000	0.0000	0.0000 0.0000		
Larger grain borer (Prostephanus truncatus)						
C	3.3a	20.0ab	26.7a	5.8a	9.2a	13.0
KF	22.7ab	17.3a	84.0b	36.8ab	12.7a	34.7
DA	43.7c	35.0b	95.7c	63.1c	53.9b	58.28
Ash	30.7b	21.0a	91.7c	50.7bc	27.6a	44.34
P value	0.0004	0.0174	0.0000	0.0024	0.0000	
Lesser grain borer (Rhizopertha dominica)						
C	0.0a	3.3a	1.7a	12.4a	3.4a	4.16
KF	11.2a	17.7a	42.6a	68.4a	27.0a	33.38
DA	25.4b	82.4c	91.6c	94.1b	97.6c	78.22
Ash	17.1ab	57.2b	64.1b	61.4a	80.7b	56.1
P value	0.0169	0.0000	0.0000	0.0000	0.0000	

NB: Each datum is a mean of 15 dose reps.
Column numbers followed by same letter were not statistically different at 95% level (DMRT).

Table 3. *Percent weight loss caused by three storage insect pests on maize/wheat treated with three inert dusts*

Sitophilus zeamais					
Post treatment storage period (weeks)					
Treatments	2	4	8	12	Mean
DA	2.12a	3.89a	3.66a	4.38a	3.51
Ash	2.65b	4.55b	4.22a	4.75a	4.04
KF	3.07b	5.10c	4.96c	5.76b	4.72
C	6.93c	12.33c	9.20c	21.27c	12.43
P value	0.0000	0.0000	0.0000	0.0000	
Prostephanus truncatus					
DA	4.55a	3.69a	4.35a	6.50a	4.77
Ash	8.64b	4.57b	5.07b	9.94b	7.06
KF	10.31c	4.28ab	5.32b	13.72c	8.41
C	12.23c	7.17c	5.67b	12.40bc 9.37	
P value	0.0000	0.0001	0.0069	0.0000	
Rhizopertha dominica					
DA	1.85a	3.07a	2.47a	2.04a	2.36
Ash	2.23ab	3.53b	2.73bc	2.58ab	2.76
KF	2.30b	3.79b	2.69b	3.27b	3.01
C	3.13b	4.87c	3.00c	5.87c	4.22
P value	0.0222	0.0000	0.0017	0.0000	

Column means followed by same letter were not statistically different at P=0.05
Each datum is a mean of 15 dose levels

Table 4. *Effect of inert dusts applied on maize or wheat on the emergence of F_1 progeny of three storage insect pests after 14 day exposure*

Pest	**Applied treatments**				
	DA	**Ash**	**KF**	**Control**	**P value**
S. zeamais	6.87a	17.07b	29.73c	148.0d	0.0000
P. truncatus	18.6a	65.93b	87.27b	138.0c	0.0000
R. dominica	1.13a	2.0a	2.13a	0.7a	ns

Each datum is a mean of 15 observations
Row numbers followed by same letter were not statistically different at P=0.05.

Acknowledgement

This study could not have progressed to fruitful conclusion without the intervention or participation of certain persons and institutions. The authors would like to sincerely acknowledge the drive and support by the African Diatomite Industries Limited (ADIL) who, initiated the Kensil evaluation process to widen its use from beverage and paint industries to include agriculture. Special mention goes to Mr. Philip Chesang for actively monitoring the progress of the work at all project sites and ensuring continuous project funding which is greatly acknowledged. Many thanks go to the KARI Management through our Centre Director for creating conducive environment under which the work was carried out. The authors greatly appreciate those others who cannot be named for playing different roles.

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