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RESEARCH ARTICLE

Efficacy of chlorpropham (CIPC) alternatives on suppressing sprouting of ware potato tubers stored at ambient tropical temperatures

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ABSTRACT

High postharvest losses are a major constraint to long term storage of potato in Kenya. Cold storage is not feasible due to the high cost and lack of facilities. Use of sprout suppressants could be an attractive proposition. However, sprout-preventing chemicals, such as Chlorpropham (CIPC) negatively impact the environment and human health. This study evaluated the efficacy of two alternative sprout suppressants, Peppermint oil (PPO) and 1, 4 Dimethylnaphthalene (DMN) using three potato cultivars with varying dormancy stored under ambient conditions. After 24 weeks of storage, CIPC suppressive effects was 100% on the three cultivars whereas the non-treated tubers had 100% sprout emergence by sixth (short dormancy), twelfth (medium dormancy), fourteenth (long dormancy) weeks of storage. Inhibition by DMN and PPO varied with cultivars, but were significantly ($P<0.05$) better than the non-treated tubers. PPO inhibited sprout development for 18 weeks on medium and long dormancy cultivars and 14 weeks on short dormancy cultivar. DMN treatments did not prolong storage in short and medium dormancy cultivars but it greatly reduced sprout growth but not as effective as peppermint. In long dormancy cultivar, DMN equalled the effectiveness of CIPC. The study showed that DMN and peppermint oil could be effective sprout suppressant alternatives to CIPC under tropical ambient conditions and useful for resource-constrained potato farmers.

Keywords: Dimethylnaphthalene, peppermint oil, sprout inhibitor, tropics

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INTRODUCTION

High postharvest losses are major constraint facing the potato industry in Kenya. On average 19 per cent of total production per hectare is lost every season (Kaguongo et al., 2014). Sprouting is a major cause of quality loss in stored tubers. Sprouting leads to a rise in the rate of respiration and transpiration, remobilization of stored food reserves causing tuber shrinkage due to moisture loss, loss of tuber firmness and loss of nutritive value (Sonnewald and Sonnewald, 2014; Rezaee et al., 2011; Pinhero et al., 2009; Suttle, 2004; Teper-Bamnolker et al., 2010. Postharvest losses as a result of sprouting, rotting and weight loss are particularly high under non-refrigerated storage environments with high ambient temperatures. In the major potato producing areas of Kenya, potatoes are harvested during the warm months of January–March and August–September. Most of Kenyan popular potato varieties have a dormancy period of 1–2 months (NCPK, 2015). Due to limited post harvest storage systems, there are gluts and scarcity in market leading to huge price fluctuations. Most of the farmers are not able to store their potatoes for long for improved prices due to sprouting. Therefore, sprout management in Kenya has to take into consideration of both short-term and long-term storage at high ambient temperature storage conditions. Isopropyl N-(3-

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chlorophenyl carbamate) (CIPC) is the most commonly used sprout suppressant on potatoes (Paul et al., 2016). CIPC acts as a mitotic inhibitor by interfering with the process of spindle formation during the cell division hence effectively prevents new growth leading to sprout inhibition (Kleinkopf and Frazier, 2002). However, sprout-preventing chemicals, such as chlorpropham (CIPC), can negatively impact the environment and human health. 1, 4 and 1, 6-Dimethylnaphthalene (DMN), isomer s of dimethylnaphthalene are naturally produced compounds in potato. They have been shown to exhibit sprouting suppressing effects on potato tubers (Meigh et al., 1973). DMN mode of action is still not clear but it has been suggested that it acts by extending the natural period of dormancy through regulation of phytohormones (Beveridge et al., 1981b; Campbell et al., 2010; Kleinkopf et al. 2003). Natural compounds containing monoterpenes have also been found to be effective potato sprout inhibitors. Peppermint oil (PMO) contains monoterpenes such as menthol 40–45%, menthone 20–30% and lower concentration of methyl acetate, 1,8-cineole and pulegone (Gómez-Castillo et al., 2013; Maffei et al.,2001). Peppermint oil has also shown promising sprout inhibition capacity (Frazier et al., 2004). The mode of action of these monoterpenes in sprout control is not clear but they inhibit sprouting by causing cell membrane damage mainly at the meristem tips of the sprouts (Teper-Bamnolker et al., 2010.; Vaughn and Spencer, 1991).

Due to increasing concern for consumer health and safety, there is considerable interest in finding effective potato-sprouting suppressants that have a negligible environmental impact. Most research work on environmentally safe sprout suppressants have been done in the under cool storage conditions. There is limited information on the efficacy of these sprout suppressants under tropical environment and storage at high ambient temperatures. This study was therefore designed to evaluate the efficacy of 1, 4 dimethylnaphthalene (DMN) and peppermint oil on storage behaviour of three commercial cultivars stored under ambient temperatures in Kenya.

MATERIALS AND METHODS

Plant Material

Certified potato seeds of three commercially cultivated cultivars, Shangi (short dormancy), Asante (medium dormancy) and Kenya Mpya (medium to long dormancy) were planted April to July 2013 and October 2013 to January 2014 at Kabete Field Station of the University of Nairobi. Standard agronomic practices recommended for potatoes including ridging, pest control, fertilization and weeding were utilized. Plants were dehaulmed two weeks before harvest. Tubers were hand-harvested and sorted into three categories: Tubers were hand-harvested and sorted into three categories: ware (table stock 50-90 mm diameter), seed (30-50 mm diameter), and rejects (<25 mm diameter). Healthy, medium-sized ware tubers above 55mm in diameter; free of any evident disease and without any signs of sprouting were selected and cured for 14 days under ambient conditions before applying the treatments. The freshly harvested potato tubers were sorted out and healthy tubers above 55mm in diameter; free of any evident disease and without any signs of sprouting were selected and cured for 14 days under the prevailing ambient conditions. The potato tubers were then subjected to sprout suppressant treatments and stored for 24 weeks.

Treatment Application

Commercial peppermint oil (*Mentha piperita)* (Sigma-Aldrich, USA CAS-No.: 8006-90-4) was applied to tubers using the wick method as described by Frazier et al. (2004) at a rate of 50 ppm per sample applied after every two weeks for 24 weeks. Twenty potato tubers were put into khaki bags then wrapped with plastic bags to prevent the mint oil from venting out. Pepper mint was applied in vapour form by placing a filter paper impregnated with peppermint oil in the bags containing potato tubers.

The filter paper was between the khaki bag and the polyethylene bag so that no direct contact with the tubers occurred. A control experiment was conducted in the same conditions as the peppermint oil experiments although without any treatment.

For Isopropyl N-(3-chlorophenyl carbamate) chloropropham (CIPC), and 1,4- Dimethylnaphthalene (DMN) application, tubers were thinly spread on plastic trays and CIPC (granules containing 95% CIPC), at a rate of 22 mg a.i. Kg $^{-1}$. (Sigma-Aldrich, USA), or DMN applied at the rate of 100 mg a.i. Kg $^{-1}$ of fresh tuber weight as a liquid fog (Sigma-Aldrich, USA). The controls (non-treated tubers) were sprayed with distilled water. After treatment, the tubers were wrapped in airtight plastic bags for 24 h. The treated potato tubers (20 tubers per cultivar per treatment) in each replicate were packed in khaki (cloth) bags. Potato tubers were stored for up to 24 weeks at ambient storage of 23 C. The experiment had three replications per treatment.

Data collection

Dormancy and sprouting parameters were assessed following the procedure as specified in Shibairo et al., 2006. Dormancy was considered to have ended when over 80% of the tubers in a sample had sprouts ≥ 3 mm long. Sprouting was measured by evaluating samples of 20 tubers in three replicates weekly up to 6 months of storage. Sprouting percentage was calculated as the percentage of the number of sprouted tubers in the sample. Weight loss was calculated as the change between the initial weight at the beginning of the experiment and the final weight measurements at the end of storage. The change in weight was expressed as a percentage of the initial weight. Data was also taken on dormancy period, sprout length, number of sprouts per tuber, and sprout thickness.

Data analysis

Prior to analysis, percentage data were subjected to tests for normality of variances. In cases where the assumptions of normality and equality variances were not met, the data were subjected to square root transformation to correct these problems prior to analysis. All data were subjected to analysis of variance using Genstat software (Pyne et al. 2009; v. 15, VSN, UK, 2010) with duration of storage, treatments and replicates as factors and % sprouting, sprouts number, sprout length, sprout thickness and weight loss as variables. In case of significant differences, comparisons among treatment means were computed by using Tukey's least significant difference test at 5%. The association among the different components of sprout growth and weight loss was assessed using correlation analysis.

RESULTS

Effect of suppressants on dormancy period and % sprouting

Application of CIPC, DMN and pepper mint significantly increased dormancy period of the three cultivars compared to the control (Table 1). In all treatments, cultivar Shangi tubers had the shortest dormancy period while Kenya Mpya had the longest dormancy period (Table 1). Sprouting was not observed 18 weeks after CIPC treatment while DMN treatments maintained complete sprout inhibition for 16 weeks after treatment for cultivar Kenya Mpya. However, in cultivar Asante, DMN had a moderate inhibitory while for Shangi it had no effect with treated tubers beginning to sprout at the same time with control treatment (Table 1).

Table 1. Effect of sprout suppressant treatment on dormancy of 3 potato cultivars during storage at ambient temperatures

	[a]Dormancy period (days)		
	Cultivars		
Treatment	**Asante**	**Kenya Mpya**	**Shangi**
Control	60[e]	70[d]	28[f]
CIPC	140[ab]	146[a]	133[b]
DMN	70[d]	98[c]	28[f]
PPO	98[c]	98[c]	56[e]

CIPC = Isopropyl N-(3- chlorophenyl carbamate) chloropropham, DMN = 1,4 Dimethylnapthalene, and PPO = Pepper mint essential oil. [a]Dormancy refers to number of days from harvest to sprout development (sprouts ≥3 mm long) in 80% of tubers . Means in the rows and columns of the foliar application experiment with different letters indicate significant differences ($P < 0.05$) in treatment means based on Tukey's Significant Difference.

CIPC treated tubers had a 23.3%, 16.7% and 10% of sprouting for cultivars Shangi, Asante and Kenya Mpya respectively at the end of the 24 weeks storage period (Table 2). DMN treatments did not prevent the onset of sprouting in cultivars Shangi and Asante but it greatly reduced the rate of sprouting compared to the control treatment (Table 2). Sprouting was complete on control treatments at the end of week 4 and 10 for cultivars Shangi and Asante respectively. DMN was effective only on cultivar Kenya Mpya as compared to Shangi and Asante cultivars. After 24 weeks of storage, sprouts developed in 100% of the non-treated tubers from the three cultivars. Overall, the suppression of sprouts (sprout inhibition) varied with the types of sprout inhibitor treatments, storage duration and tuber cultivars. Overall, sprout development increased with increased of storage period (weeks) (Table 2).

Effect of suppressants on sprout numbers per tuber and sprout length

The number of sprouts per tuber was significantly ($P < 0.05$) influenced by the interactions of sprout suppressant treatment, cultivar and storage period. The non-treated tubers had the highest number of sprouts per tuber compared to CIPC, DMN and PPO treatments throughout the experimental period (Table 3). There was no difference in number of sprouts per tuber between CIPC and DMN treatment for cultivar Kenya Mpya. In treatments with DMN, the sprout numbers increased initially then decreased towards the end of the storage duration in tubers of cultivars Asante and Shangi. CIPC treatments produced the fewest number of sprouts per tuber and at the end of the experiment all cultivars had less than 0.3 sprouts per tuber (Table 3). In DMN treatment, the number of sprouts increased initially then started to decline towards the end of the experiment in cultivar Asante and Shangi tubers (due to necrotic sprouts falling off). CIPC treatments produced the lowest number of sprouts per tuber and at the end of the experiment all treatments had less than 0.3 sprouts per tuber (Table 3).

Table 2. Effect of sprout suppressant treatment on tuber sprouting (%) of 3 potato cultivars and non-treated tubers stored atambient temperatures

		Storage duration (weeks)											
Treatment[x]	**Cultivar**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**
Non-treated	Asante	0	0	0	0	80	100	100	100	100	100	100	100
CIPC		0	0	0	0	0	0	0	0	0	3.3	6.6	16.7
DMN		0	0	0	0	60	63.3	63.3	63.3	63.3	63.3	63.3	63.3
PPO		0	0	0	0	0	0	0	26.7	20	20	20	26.6
Non-treated	Kenya	0	0	0	0	80	96.7	100	100	100	100	100	100
CIPC	Mpya	0	0	0	0	0	0	0	0	0	3.3	6.7	10
DMN		0	0	0	0	0	0	0	3.33	6.67	13.3	16.7	20
PPO		0	0	0	0	0	0	0	30	33.3	23.3	20	26.6
Non-treated	Shangi	0	93.3	100	100	100	100	100	100	100	100	100	100
CIPC		0	0	0	0	0	0	0	0	0	10.0	13.3	23.3
DMN		0	3.3	16.7	73.3	76.7	76.7	76.7	76.7	76.7	76.7	76.7	76.7
PPO		0	0	0	0	46.7	36.7	26.7	23.3	16.7	20.0	23.3	30.0
Means		0	8.5	9.7	14.4	36.9	34.4	38.8	43.6	55.6	44.4	45.6	49.4
Std Error		0	26.8	28.8	34.2	40.5	45.1	45.1	41.8	54.2	40.3	39.2	35.9
LSD(0.05)		ns	7.14	7.41	8.07	8.78	9.27	9.27	8.92	10.16	8.76	8.64	8.27

[x]CIPC = Chlorpropham, DMN = 1,4-Dimethylnapthalene, and PPO = peppermint essential oil; Std Error = Standard error of the mean.

Table 3. Effect of tuber applied sprout suppressants Chloropropham (CIPC), 1,4-Dimethylnapthalene (DMN), and pepper mint essential oil (PPO) on the number of sprouts per tuber in three potato cultivars stored at ambient temperature

		Number of sprouts per tuber										
		Storage duration (weeks)										
Treatment	**Cultivar**	2	4	6	8	10	12	14	16	18	20	22
Control	Asante	0	0	0	0	1.3	1.8	3.0	4.2	5.6	6.3	7.9
CIPC		0	0	0	0	0	0	0	0	0	0.0	0.1
DMN		0	0	0	0	1.8	1.8	3.3	4.4	4.9	4.8	4.1
PMO		0	0	0	0	0	0	0	0.1	0.8	0.6	0.6
Control	Kenya Mpya	0	0	0	0	1	3.2	5.2	7.6	8.6	12.3	13.5
CIPC		0	0	0	0	0	0	0	0	0	0.1	0.1
DMN		0	0	0	0	0	0	0	0	0.13	0.3	0.4
PMO		0	0	0	0	0	0	0	2.6	2.2	1.4	0.6
Control	Shangi	0	1.8	6.7	13.1	13.4	13.7	13.9	14.0	14.3	14.5	15.0
CIPC		0	0	0	0	0	0	0	0	0	0.2	0.2
DMN		0	0.1	0.4	3.2	3.3	3.8	4.8	4.6	4.2	3.6	3.4
PMO		0	0	0	0	2.8	2	1.1	0.8	0.9	1	1.3
LSD (5%)		ns	0.27	0.54	0.51	0.66	0.61	0.77	0.70	0.59	0.91	0.69

[x]CIPC = Chlorpropham, DMN = 1,4-Dimethylnapthalene, and PPO = peppermint essential oil

All the treatment effectively suppressed sprout growth compared to the control. CIPC presented the most effective sprout growth suppressant that resulted in sprout length of 2mm, 1.67mm and 1.77mm for cultivars Shangi, Asante and Kenya Mpya respectively 24 weeks after treatment. Sprout emergence was delayed until week 20 for CIPC treated tubers in all the cultivars. As a result, the lowest sprout length was recorded for this treatment. Sprout length increased with storage time up to the end of experiment. Application of DMN maintained a sprout length of <4 mm after 24 weeks in storage. Sprouts on the

untreated tubers averaged 20mm, 18.24mm and 23.15mm in length for cultivars Shangi, Asante and Kenya Mpya, respectively at this same point

Effect of sprout suppressants on weight loss

Application of sprout suppressants significantly decreased weight loss (Figure 1). CIPC treatment compared recorded the highest prevention of weight loss compared to DMN and PMO treatments (Figure 1). There were significant correlations ($P<0.01$) between weight loss and sprout growth. Weight loss had strong positive correlation with sprout length ($r=0.907^{**}$) and the number of sprouts ($r=0.828^{**}$). Tubers with long sprout lengths lost more weight than tubers with shorter sprouts. In addition, tubers with more sprouts lost more weight than tubers with fewer sprouts.

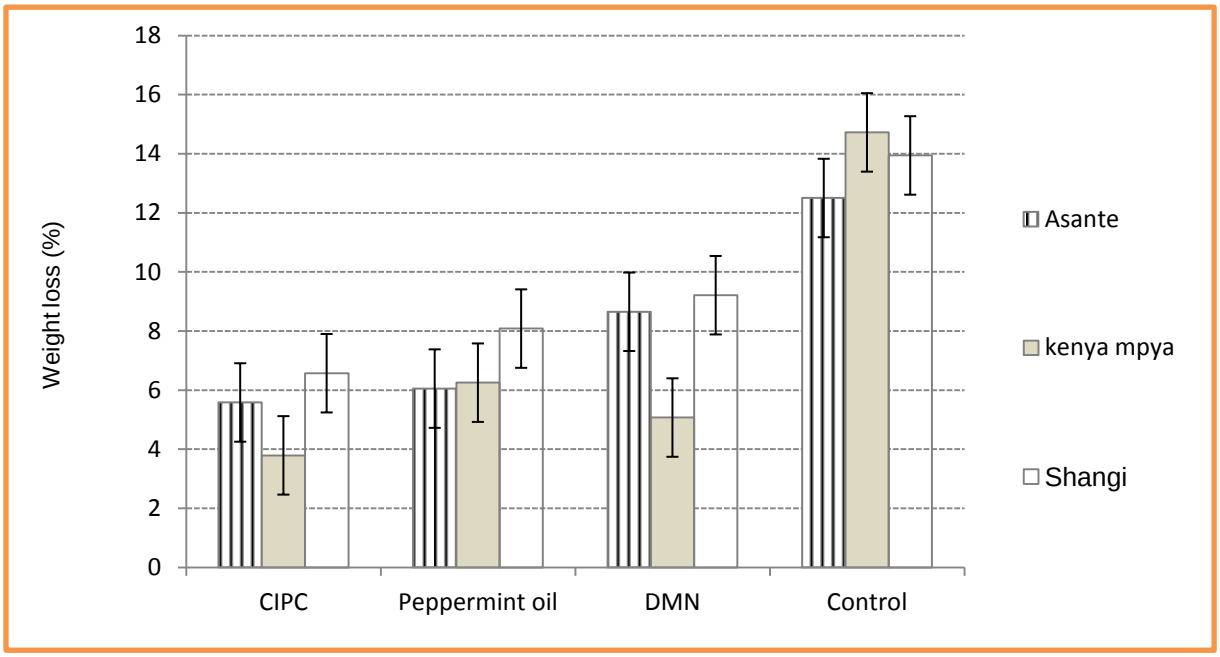

Figure 1: Weight loss (%) at the end of storage period of three potato cultivars cultivars treated with peppermint oil, DMN, and CIPC and stored at ambient temperatures in Kenya.

DISCUSSION

Significant sprout inhibition was achieved for 24 weeks under ambient storage temperatures (23^{o}C) with a single application of CIPC at a dose of 22 mg kg^{-1}). Our results are similar to previous research which showed the efficacy of CIPC on tubers (Sanli *et al*., 2010; Mehta and Kaul, 1991; Mehta et al, 2007; Mehta et al, 2010; Nyankanga et al. 2018). Tuber sprouting increased with increased storage time. This could have been as a result of decreasing suppressant residue levels within the tuber beyond the level necessary for complete sprout inhibition with time. Storage time has been shown to have a substantial reduction on the CIPC residue present in the potato tuber (Mondy et al., 1992; Mehta et al., 2010; Sakaliene et al., 2008; Lewis et al., 1997). The authors have attributed the decrease in CIPC residue over time to microbial breakdown. Similarly, DMN residue levels within tubers have been reported to decrease with increasing storage time (Lewis et al., 199; De Weerd et al., 2010). Previous research indicated some toxicity attributed to CIPC application on potato tubers (Mondy *et al*. 1992). The same authors reported that tubers treated with CIPC and stored at 5 C contained higher residue levels than those stored at 20

C. This implied that the residue levels of CIPC varied with storage duration, as the high concentration in tubers immediately after treatment subsequently decreased with storage time (Singh and Ezekiel, 2010; Sakaliene *et al*., 2008). In this research, we did not assess the residue levels of CIPC on potato tubers, but hypothesize that due to the longer duration of storage (>20 weeks), residue levels may be quite low (Mehta and Ezekiel, 2002).

Tubers treated with DMN had nearly 80% sprout development at the end of 24 weeks storage. In contrast, PPO showed better suppressive activity of sprout development DMN, implying that it could be more useful to resource-constrained farmers due to its cost effectiveness, rapid recovery through distillation process and ease of applications. The efficacy or essential oil derived from peppermint for sprout inhibition on potato has been previously documented (Xin et al. 2008). It has been demonstrated that essential oil (derived from plants) whose composition consisted mainly of monoterpenes and carvone were more effective than CIPC in reducing sprouting, tuber weight loss and tuber rot over a 225-day storage time (Alamar et al. 2017). However, due to the volatility of essential oils, it has been suggested that multiple applications are needed for inhibition of sprout development on tubers. The mode of action of the essential oils were postulated to be associated with disruption of cell membranes of microbes. Furthermore, it has been shown that some essential oil contains compounds of the unsaturated ketone group which inhibits sprout growth (Capelle et al. 1996). Other mechanisms of essential oils have been associated with degradation of enzymes that are crucial for the biosynthesis of cytokinins, gibberellic acid and abscisic acids, and membrane components of the cell, thereby affecting sprout development.

DMN showed variable sprout suppression among the three cultivars. Variability of sprout suppression with the DMN treatments among the cultivars may have been due to genetic differences. De Weerd et al. (2010) tested DMN critical residue levels necessary for suppressing sprout development on three cultivars 'R. Norkotah', 'R. Burbank' and 'Shepody'. In order to maintain sprout control, tuber residues were 2.7 ppm for cultivar 'R. Norkotah', 1.6 ppm for cultivar 'Shepody' and 1.4 ppm for cultivar 'R. Burbank'. This is an indication that different cultivars require different tuber residue levels to suppress sprouting in storage. DMN applied at the rate of 100 mg kg^{-1} maintained a sprout length of <4 mm at the end of 24 weeks in storage while sprouts on the untreated tubers averaged 20mm, 18.24mm and 23.15mm in length for cultivars Shangi, Asante and Kenya Mpya respectively. Similar results have been reported previously (Lewis et al, 1997; Knowles et al., 2005). Observations of DMN treated tubers from all the cultivars revealed that developing sprouts tips turned black, necrotic after some time in storage. Similar observations were made by Lewis et al., 1997.

Potato tubers treated with sprout suppressants lost less than 10% of their fresh weight and therefore they did not suffer sufficient moisture loss to affect their physical appearance, and consequently, the tubers could be sold for prices comparable to that of freshly harvested tubers. The significant reduction in weight loss observed in tubers resulting from treatment with suppressants could be associated with late sprouting and few numbers of sprouts. Weight loss showed a significant positive correlation with number of sprouts and sprout length. Alexopoulos et al. (2008) reported that sprouting results in increased metabolic activity due to sprout growth leading to increased respiration and weight loss. Sprouting causes direct weight loss due to their faster metabolic activity, high surface area and increased respiration resulting in increased starch breakdown leading to weight loss (Gautam et al., 2013; Benkeblia et al., 2008; Wustman and Struik, 2007). Additionally, the presence of sprouts increases evaporation because the epidermis of the sprouts is 100 times more permeable to water than the tuber periderm (Benkeblia et al., 2008). Significant differences in weight loss among the cultivars were observed. Cultivar Shangi treated and untreated tubers recorded higher weight loss than Asante and Kenya Mpya. Additionally, cultivar Kenya Mpya tubers recorded significantly lower weight loss. Pande et al. (2007) reported that tuber weight loss was cultivar dependent and the cultivars that exhibit longer dormancy with reduced sprout growth and less number of sprouts has restricted weight loss. Ezekiel et al. (2004) indicated that variations in weight loss during storage among cultivars are due to either their periderm

characteristics or their sprouting behavior.

CONCLUSION

CIPC provided the best suppression of sprout development and elongation, indicating that it has the greatest suppressive activity. DMN and Peppermint oil also provided comparatively good sprout inhibition indicating that they could be useful for resource-constraint potato farmers. As there is paucity of information on effects of sprout inhibitors on tropical-adapted potato cultivars and storage at high ambient temperatures, the findings of this research could provide much needed data for optimum potato storage and utilization in the highland tropics.

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