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Binary mixture efficacy of NeemAzal and *Plectranthus glandulosus* leaf powder against cowpea and maize weevils

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Abstract

The aim of this study was to determine the insecticidal efficacy of mixture of NeemAzal a commercial neem product and *Plectranthus glandulosus* leaf powder against *Callosubruchus maculatus* and *Sitophilus zeamais*. Mixed at various proportions (100 + 0, 75 + 25, 50 + 50, 25 + 75 and 0 +100%, these powders were tested on adult mortality, inhibition of offspring production and their persistence on *C. maculatus* and *S. zeamais*. All the mixed NeemAzal and *P. glandulosus* caused significant mortality to adult *C. maculatus* and *S. zeamais*. No significant difference was observed among the mixed powders that were subjected to the three mixture proportions regarding the mortality they caused to the weevils. The mixed 75% NeemAzal + 25% *P .glandulosus* of powder led to a higher mortality (100%) of both insect species, three (5 g/kg) days post exposure. The three days LC_{50} values decreased with ascending proportion of NeemAzal in the mixture from 3.21 g/kg (25% NeemAzal + 75% *P. glandulosus*) to 0.24 g/kg (75% NeemAzal + 25% *P. glandulosus*) in *S. zeamais*. In *C. maculatus,* the opposing effect was observed. The number of F_1 progeny produced reduced significantly ($P \leq 0.01$) in both insect species with the mixture proportion of botanicals. The mixtures reduced better the adult progeny production than the botanicals applied alone. The 75% *P. glandulosus* + 25% NeemAzal persisted well on grains up to 180 days for all dose levels. Powder from NeemAzal and *P. glandulosus* leaves stand as good candidates to protect maize and cowpea against the infestation of *S. zeamais* and *C. maculatus* respectively during storage. Mixing these products could not be advantageous since the binary mixture gave similar result as when they were applied alone.

Key words: Callosobruchus maculatus, Sitophilus zeamais, mixture, Plectranthus glandulosus, NeemAzal, efficacy

Introduction

Crop production plays major importance in the livelihood of people all over the world and particularly in developing countries where it is considered as the principal activity on which most economies depend on (Gustavsson et al., 2011. Paradoxically many farmers loss heavy quantity of cultivated plants because of the attack by insect pests. *Sitophilus zeamais* and *Callosobruchus maculatus* are the most important pests stored maize and cowpea respectively with great losses mostly in tropical countries(Guèye et al., 2011). To reduce post-harvest losses, different methods of grain protection are used by small holder farmers as well as at the industrial level (Isman, 2006). However, over the past decades, synthetic chemical insecticides have played a significant role in modern agricultural pest management (Guo *et al.,* 2014). Their repeated use over the years has led to the evolving of resistance in pest populations and fostered environmental and human health concerns (Ofuya, 2003). These problems have highlighted the need for the development of new types of selective insect-control alternatives (Lee et al., 2001), which combine broad spectrum action against stored product insect pests with low toxicity to non-targeted organisms, but at the same time also readily available and affordable to the small-scale grower (Nukenine et al*.,* 2007). Nowadays, products from plant origin are recommended as alternative to the hazardous synthetic chemicals. *Plectranthus glandulosus* leaf powder and essential showed greater insecticidal efficacy against adult insects of stored cereals (Nukenine et al*.,* 2011).The commercial NeemAzal powder which is constituted of the mixture of diatomaceous earth with azadiracthin was effective against the maize weevil (Nukenine et al., 2011). To promote the use of safer NeemAzal or *P. glandulosus* leaf powder combined with good efficacy in stored product protection, the mixture of these powders to reduce the quantity applied need to be reconsidered. The aim of the present work is to determine the insecticidal efficacy of mixture of NeemAzal a commercial neem product and *P. glandulosus* leaf powder against *C. maculatus* and *S. zeamais***.**

Material and Methods

The leaves of *P. glandulosus* were collected around Ngaoundere (Quartier Champ de prière) (latitude 7°22′ North and longitude 13°34′ East, altitude of 1,100 m.a.s.l.), located in the Adamawa region of Cameroon. The plants were less than one-year old and only the green leaves were harvested from plants which were yet to attain the flowering stage. The collected leaves were sun-dried (29 ± 4°C). Dried leaves were hand crushed. The crushed leaves were ground into powder using a Bosch Universal grinder (model MUM 6012, Remscheid, Germany) until the particles passed through 0.1 mm mesh sieve. NeemAzal powder, was provided by Trifolio-M GmbH, Lahnau, Germany.

The parent adults of *S. zeamais* and *C. maculatus* were obtained from colonies maintained at JKI, Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Berlin since 1968 and 2011, respectively. *S. zeamais* were reared on maize (Ricardino variety) while *C. maculatus* were reared on cowpea (Black eyes Perou).

NeemAzal powder were mixed with *P. glandulosus* leaf powder in the proportions of 100/0, 75/25, 50/50, 25/75 and 0/100% in glass jars. Each glass jar was shaken with a bidimensional mixer (Gerhardt, Dreieich, Germany) for 5 hours to ensure uniform mixture of the powders. The masses 0.125, 0.25, 0.5, 0.75 and 1 g were separately introduced to 50 g of maize or cowpea in 250 ml glass jars to give the doses of 2.5, 5, 10, 15 and 20 g/kg of maize or cowpea. Controls consisted of grains devoid of the plant powders. Each binary mixture was tested on *S. zeamais* and *C. maculatus* for adult toxicity, progeny production. The persistence test was performed with 75% *P. glandulosus* + 25% NeemAzal.

The co-toxicity coefficient of powder mixture was used to determine their responses: A co-toxicity coefficient of less than 80 is considered as antagonistic, between 80 and 120 as additive, and higher than 120 as synergistic (Sun and Johnson 1960; Islam *et al.,* 2010). If a mixture (M) compounds of two parts (A and B), and both components have LC_{50}, then the following formulas are used (A serving as standard):

Toxicity index (TI) of A = 100

Toxicity index (TI) of B = $\frac{\text{LC50 of A}}{\text{LC50 of B}} \times 100$

Actual TI of Mixture = $\frac{\text{LC50 of A}}{\text{LC50 of M}} \times 100$

Theoretical TI of M = TI of A × percentage of A in M + TI of B × percentage of B in M

Co-toxicity coefficient = $\frac{\text{Actual TI of M}}{\text{Theoretical TI of M}} \times 100$

If one component of the mixture alone (for example B) causes low mortality at all doses (< 20%), then the co-toxicity coefficient of the mixture was calculated by the formula: Co-toxicity coefficient = LC_{50} of A alone/LC_{50} of A in the mixture × 100.

Data on % cumulative corrected mortality and % reduction in F_1 progeny were arcsine [(square root(x/100)] transformed and the number of F_1 progeny produced were log (x + 1) transformed to homogenise the variance. The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (SAS Institute, 2008). Tukey (HSD) test (P = 0.05) was applied for mean separation**.**

Results

All the different combinations of NeemAzal and *P. glandulosus* generally caused significant mortality to adult *C. maculatus* and *S. zeamais* compared to the control (Tables 1and 2). The increase in mortality with ascending dose levels and time exposure was much more pronounced within three days post exposure than thereafter, irrespective of mixture proportions and insect species. Overall, mixture proportions generally had no effect on the mortality of the two insect species caused by the mixed *P. glandulosus* leaf powder and NeemAzal. However, the combination 25% NeemAzal + 75% *P .glandulosus* powder tended to be less potent to both insect species, since the lowest tested powder dose of 2.5 g/kg caused lower than 100% mortality to *C. maculatus* (6-d) and *S. zeamais* (7-d) for this combination while the other two combination proportions caused complete mortality . The highest tested dose (20 ml/kg) achieved complete mortality of both weevils three days post exposure for all mixtures, except the 25% NeemAzal + 75% *P. glandulosus* leaf powder which caused a maximum mortality of 87.50% in *S. zeamais*.

Table 1: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* exposed to binary combinations of NeemAzal and *Plectranthus glandulous* leaf powder

Doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)†					
	100% *P. gland*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(4, 15)}$ ‡
			3-d			
0	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
2.5	0.00 ± 0.00^{bC}	77.50 ± 2.23^{bB}	86.25 ± 2.39^{bAB}	93.75 ± 1.25^{bA}	87.50 ± 4.33^{bAB}	208.65^{***}
5	0.00 ± 0.00^{bC}	85.00 ± 2.89^{bcB}	97.50 ± 2.50^{aA}	100 ± 0.00^{aA}	96.25 ± 2.39^{abA}	143.47^{***}
10	0.00 ± 0.00^{bC}	96.25 ± 2.39^{abAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	91.25 ± 2.39^{abB}	264.67^{***}
15	3.75 ± 1.25^{abB}	95.00 ± 3.54^{abA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.00 ± 2.04^{abA}	102.92^{***}
20	5.00 ± 2.04^{aB}	98.75 ± 1.25^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	98.75 ± 1.25^{aA}	165.62^{***}
$F_{(5, 18)}$ ‡	5.51^{**}	222.49^{***}	795.68^{***}	6265.00^{***}	247.17^{***}	
			6-d			
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
2.5	0.00 ± 0.00^{eC}	92.50 ± 3.23^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	314.11^{***}
5	13.75 ± 1.25^{dB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	3941^{***}
10	21.25 ± 1.25^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	5310.1^{***}
15	31.25 ± 2.39^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	825^{***}
20	42.50 ± 3.23^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	690.67^{***}
$F_{(5, 18)}$ ‡	230.45^{***}	936.60^{***}	$–^{***}$	$–^{***}$	$–^{***}$	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; P < 0.05).
‡ ** P < 0.01; *** P < 0.001. *P. gland = Plectranthus glandulosus.*

The toxicity parameters of the binary mixture of *P. glandulosus* and NeemAzal powders to *C. maculatus* and *S. zeamais* are given in (Table 3). The 3-d LC_{50} values decreased with ascending proportion of NeemAzal in the mixture from 3.21 g/kg (25% NeemAzal + 75% *P. glandulosus*) to 0.24 g/kg (75% NeemAzal + 25% *P. glandulosus*) for *S. zeamais*. With *C. maculatus,* the opposite effect was observed, the LC_{50} values increased as the quantity of NeemAzal in the mixture increased. When the proportion of NeemAzal was ≥ 50% the LC_{50} and LC_{95} LC were not estimated due to complete adult mortality. The slopes seemed similar (1.24 – 1.51) for all the combinations of the powders in *S. zeamais* while they decreased (18.82 – 1.45) with increase in the quantity of *P. glandulosus* in the mixture. All the estimated co-toxicity coefficients were less than 80.

The number of F_1 progeny and the percentage inhibition of the progeny of *C. maculatus* and *S. zeamais* that emerging from grains treated with different combinations of NeemAzal and *P. glandulosus* at different doses are shown in Table 4. The number of emerging F_1 progeny reduced significantly (P ≤ 0.01) in both insect species with ascending of the botanicals. The binary combinations of the powders reduced progeny emergency more than each botanical applied alone, with NeemAzal being more potent than *P. glandulosus*. All the binary combinations of the powders completely suppressed progeny production in *S. zeamais*. On cowpea 97.77%, 97.15% and 66.78% inhibition of *C. maculatus* emergence were recorded respectively with the combinations 25% *P. glandulosus* + 75% NeemAzal, 50% *P. glandulosus* + 50% NeemAzal and 75% *P. glandulosus* + 25% NeemAzal at the highest tested dose of 20 g/kg.

Table 2: Corrected cumulative mortality (mean ± SE) of *Sitophilus zeamais* exposed to binary combinations of NeemAzal and *Plectranthus glandulous* leaf powder

Doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)†					
	100% *P. gland*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(4, 15)}$ ‡
			3-d			
0	0.00 ± 0.00^{c}	0.00 ±0.00^{d}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ± 0.00^{d}	
2.5	0.00 ± 0.00^{cC}	45.00 ± 9.35^{cB}	80.00 ± 10.80^{bA}	88.75 ± 3.15^{bA}	63.75 ± 2.39^{cB}	28.23***
5	1.25 ± 1.25^{cD}	60.00 ± 7.36^{bcC}	91.25 ± 4.27^{abAB}	97.50 ± 1.44^{aA}	80.00 ± 2.04^{bB}	65.36***
10	5.00 ± 0.91^{bcD}	73.75 ± 5.15^{abC}	100 ± 0.00^{aA}	95.00 ± 2.04^{abAB}	87.50 ± 5.20^{abBC}	56.93***
15	13.75 ± 2.39^{abC}	88.75 ± 1.25^{aB}	95.00 ± 0.00^{abAB}	98.75 ± 1.25^{aA}	93.75 ± 1.25^{aAB}	190.33***
20	21.25 ± 3.75^{aC}	87.50 ± 3.23^{aB}	97.50 ± 1.44^{abAB}	100 ± 0.00^{aA}	95.00 ± 2.04^{aAB}	96.72***
$F_{(5,18)}$ ‡	18.04***	37.24***	64.95***	525.81***	183.60***	
			7-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	
2.5	30.00 ± 3.54^{cC}	92.50 ± 4.33^{aAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	82.50 ± 2.23^{cb}	51.35***
5	76.25 ± 3.75^{bB}	98.75 ± 1.2^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.00 ± 2.04^{bA}	25.93***
10	80.00 ± 5.40^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	98.75± 1.25^{abA}	20.70***
15	97.50 ± 2.50^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	1^{ns}
20	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
$F_{(5, 18)}$ ‡	156.37***	477.74***	–***	–***	578.26***	
			14-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
2.5	51.25 ± 13.90^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	12.30***
5	85.00 ± 5.40^{bAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	7.71**
10	93.75 ± 3.75^{ab}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	2.93ns
15	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
20	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
$F_{(5, 18)}$ ‡	39.71***	–***	–***	–***	–***	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; P < 0.05).
‡ ** P < 0.01; *** P < 0.001.
P. gland = Plectranthus glandulosus.

Table 3: Toxicity of binary combinations of NeemAzal and *Plectranthus glandulosus* leaf powder at different proportions to adult *Callosobruchus maculatus*

Insects/ product proportion	Slope ± S.E	R^2	LC_{50} (95% FL)[a]	LC_{95} (95% FL)[a]	Co-toxicity coefficient
C. maculatus					
			3 days		
100% *P.gland.* + 0% NeemAzal	3.49 ± 1.57	0.69	55.80 (31.40 - 4.43E^{5})	11.00 (8.22 -17.78)	
75% *P.gland.* + 25% NeemAzal	1.45 ± 0.25	0.94	0.81 (0.27 - 1.39)	11.00 (8.22 -17.78)	0.20
50% *P.gland.* + 50% NeemAzal	3.20 ± 0.84	0.73	1.15 (0.39 -1.66)	3.76 (3.14 - 5.29)	0.07
25% *P.gland.* + 75% NeemAzal	18.82 ± 1.62	0.58	2.07	2.53	0.17
0% *P.gland.* + 100% NeemAzal	0.70 ± 0.40	0.52	0.04	10.34	
			6 days		
100% *P.gland.* + 0% NeemAzal	1.97 ± 0.26	0.97	25.01 (20.11 – 35.01)	173.84 (96.60 -471)	
75% *P.gland.* + 25% NeemAzal	19.13 ± 1.09	0.58	0.10	2.56	0
50% *P.gland.* + 50% NeemAzal£	-	-	-	-	-
25% *P.gland.* + 75% NeemAzal£	-	-	-	-	-
0% *P.gland.* + 100% NeemAzal£	-	-	-	-	-
S. zeamais					
			3 days		
100% *P.gland.* + 0% NeemAzal	2.84 ± 0.52	0.84	40.23 (29.25 – 80.77)	171.52 (84.11 -872)	
75% *P.gland.* + 25% NeemAzal	1.51 ± 0.19	0.97	3.21 (2.30 - 4.05)	39.22 (26.90 - 71.3)	1.57
50% *P.gland.* + 50% NeemAzal	1.33 ± 0.43	0.72	0.51	8.90	5.17
25% *P.gland.* + 75% NeemAzal	1.24 ± 0.34	0.72	0.24 (0.01 - 0.74)	5.13 (3.20 - 8.13)	7.63
0% *P.gland.* + 100% NeemAzal	1.42 ± 0.31	0.96	1.39 (0.69 – 2.07)	19.68 (14.14 -34.30)	
			7 days		
100% *P.gland.* + 0% NeemAzal	1.45 ± 0.25	0.94	3.56 (1.04 – 5.66)	13.02 (8.05 -107.92)	
75% *P.gland.* + 25% NeemAzal	2.95 ± 1.08	0.72	0.81 (0.03 - 1.45)	2.96 (2.05 - 4.20)	2.75
50% *P.gland.* + 50% NeemAzal£	-	-	-	-	-
25% *P.gland.* + 75% NeemAzal£	-	-	-	-	-
0% *P.gland.* + 100% NeemAzal£	2.45± 0.49	0.82	1.05 (0.45 – 1.56)	4.92 (3.97- 6.75)	-

[a] FL = Fiducial limits; £ Toxicity parameters were not determinate due to 100% mortality

Table 4: Progeny production of *Callosobruchus maculatus* and *Sitophilus zeamais* in grains treated with binary combinations of *Plectranthus glandulosus* leaf powder and NeemAzal

Insects/ doses (g/kg)	Proportion of powders in mixture					
	100% *P. glandulosus*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(5, 19)}$ ‡
Number (mean ± SE) of F_1 adult progeny †						
C. maculatus						
0	443.50 ± 15.61^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	0.02^{ns}
2.5	409.50 ± 3.01^{aA}	299.75 ± 36.67^{abB}	35.25 ± 1.65^{bD}	30.75 ± 3.88^{bD}	97.00 ± 8.60^{bC}	146.42^{***}
5	355.75 ± 13.77^{abA}	250.50 ± 43.84^{bcA}	27.50 ± 1.71^{bcB}	20.50 ± 3.77^{bcB}	58.00 ± 12.46^{bcB}	68.11^{***}
10	283.75 ± 31.76^{abA}	201.50 ± 23.06^{bcA}	18.00 ± 4.04^{cdB}	18.00 ± 2.08^{bcB}	35.50 ± 8.53^{cdB}	69.85^{***}
15	260.25 ± 50.34^{abA}	144.50 ± 13.32^{cB}	12.75 ± 1.55^{dC}	11.00 ± 2.27^{cC}	17.25 ± 4.17^{dC}	44.10^{***}
20	206.50 ± 56.16^{bA}	146.00 ± 5.48^{cA}	12.50 ± 1.55^{dB}	9.75 ± 2.17^{cB}	11.75 ± 1.49^{dB}	21.26^{***}
$F_{(5,18)}$ ‡	4.43^{**}	15.84^{***}	647.93^{***}	407.35^{***}	143.42^{***}	
S. zeamais						
0	51.25 ± 0.95^{aB}	69.25 ± 2.10^{aA}	62.75 ± 1.65^{aA}	63.75 ± 2.95^{aA}	65.25 ± 2.53^{aA}	9.79^{**}
2.5	$36.75 \pm 2.25^{a A}$	0.75 ± 0.75^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	6.00 ± 0.71^{bB}	194.11^{***}
5	22.00 ± 4.71^{bA}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	4.50 ± 1.32^{bcB}	40.93^{***}
10	6.00 ± 0.91^{cA}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	1.25 ± 0.48^{bcB}	41.44^{***}
15	3.00 ± 0.71^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{cB}	28.37^{***}
20	1.75 ± 1.03^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	2.92^{ns}
$F_{(5,18)}$ ‡	63.83^{***}	570.87^{***}	1442.77^{***}	465.57^{***}	277.34^{***}	
Percentage (mean ± SE) reduction in adult emergence relative to control †						
C. maculatus						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
2.5	7.88 ± 3.21^{cdC}	31.84 ± 8.08^{cB}	91.97 ± 0.35^{cA}	92.99 ± 0.93^{bA}	77.84 ± 2.26^{cA}	73.68^{***}
5	19.35 ± 4.99^{bcC}	3.00 ± 9.96^{bcB}	93.71 ± 0.51^{bcA}	95.34 ± 0.85^{abA}	86.76 ± 2.96^{bA}	47.23^{***}
10	36.24 ± 6.45^{abC}	56.16 ± 5.17^{abA}	95.83 ± 1.04^{abB}	95.93 ± 0.37^{abB}	91.75 ± 2.17^{abB}	60.26^{***}
15	41.93 ± 10.52^{abC}	67.28 ± 2.01^{aB}	97.11 ± 0.24^{aA}	97.46 ± 2.27^{abA}	96.00 ± 1.07^{aA}	38.86^{***}
20	54.18 ± 12.18^{aB}	66.78 ± 0.74^{aB}	97.15 ± 0.35^{aA}	97.77 ± 0.58^{abA}	97.31 ± 0.37^{aA}	20.07^{***}
$F_{(5, 18)}$ ‡	13.11^{***}	35.05^{***}	1947.02^{***}	1245.03^{***}	338.90^{***}	
S. zeamais						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	$0.00 \pm 0.00d^{d}$	
2.5	28.14 ± 4.90^{cC}	98.96 ± 1.04^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	90.68 ± 1.43^{cB}	148.34^{***}
5	56.71 ± 9.53^{bC}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{a}	93.21 ± 1.89^{cB}	41.78^{***}
10	88.30 ± 1.78^{aC}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	98.05 ± 0.78^{bB}	44.25^{***}
15	94.08 ± 1.43^{aB}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	45.92^{***}
20	96.65 ± 1.96^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	2.98^{ns}
$F_{(5,18)}$ ‡	86.50^{***}	922.23^{***}	∞^{***}	∞^{***}	579.71^{***}	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; P < 0.05).
‡ ns P > 0.05; *** P < 0.001.
P. gland = Plectranthus glandulosus.

Figure 1 shows the results of the persistence of the mixture of 75% *P. glandulosus* + 25% NeemAzal on *C. maculatus* and *S. zeamais*. The efficacy of the mixture varied significantly (P < 0.001) with the ascending dose but not with the storage interval as the efficiency persisted up to the 180-d storage interval. For this binary combination, the mortality caused to *S. zeamais* and *C. maculatus* at the 180-d storage interval did not differ from the observed mortality at the 0-d storage interval (P > 0.05) for all the dose levels.

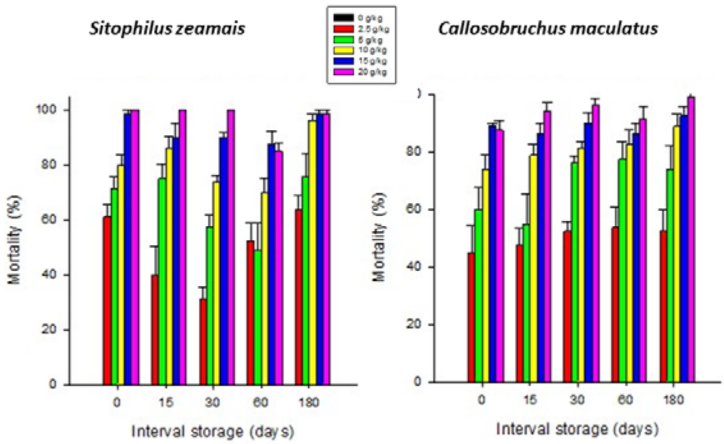

Figure 1: Corrected cumulative mortality of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed in grains treated with the combinations of *Plectranthus glandulosu*s with NeemAzal powders at different storage intervals

Discussion

The mixture of NeemAzal and *P. glandulosus* leaf powder was also antagonistic regarding the mortality they caused to *C. maculatus* and *S. zeamais*. In isolation, NeemAzal caused greater mortality to both insects than *P. glandulosus*. The mixture of *Vernonia amygdalina* and neem powder was antagonistic with respect to insecticidal efficacy (Akunne et al., 2013). The NeemAzal used in the present study was produced by incorporating azadirachtin into silica gel. The mortality observed with NeemAzal could largely be due to the presence of silica gel compared to that of azadirachtin (Ogemah, 2003). Silica gel acts by desiccation, as the insects move through grains, they pick up the powder on their cuticle which leads to the absorption of the cuticular waxes from the epicuticule surface of the insect, thus enhancing the rate of desiccation (Prasantha, 2003). Ulrich and Mewis (2000) showed that combinations of diatomaceous earth (Fossil shield (1 gm/kg) and a commercial neem product NeemAzal (1 gm/kg) resulted in higher mortality of the weevils. Since NeemAzal contains silica gel, the mixture of this powder with Fossil shield implies the doubling of the concentration of diatomaceous earths, which resulted in higher mortality in the study of Ulrich and Mewis (2000).

It could also be concluded that the binary mixtures at different proportion levels of the powders from NeemAzal and *P. glandulosus* has various effects on adult emergence. the mixture of NeemAzal and *P. glandulosus* reduced almost completely the emergence of adult insects when the rate of NeemAzal ≥ 25%. Nukenine et al., (2011) reported that under fluctuating conditions, NeemAzal powder registered similar results on *S. zeamais*. It seems that the silica gel absorbed the water contained in grains which affected the development of the weevils. Before treatment the moisture content of the grains was above 12% and after F_1 progeny evaluations, this value decreased to less than 10%. When the moisture content of the grains is less than 10%, the development of immature stages of both insect species is hindered.

NeemAzal contains silica gel and for this reason, the activity of its mixture with *P. glandulosus* was more or less constant up to 180 d compared to the 70% reduction in the efficacy of *P. glandulosus* alone. Silica gel is an inert durst and does not contain volatiles like *P. glandulosus,* which loses its active ingrediants with time. The activity of *Ocimum basilicum,* an aromatic plant of the Lamiaceae family like *P. glandulosus on S. zeamais* mortality declined most 0 (80% mortality) and 28 d (15%

moratlity) (Mwangangi and Mutisya, 2013), which is in conformity with the results of the present work.

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Effects of chlorpyrifos-methyl and pirimiphos-methyl applied with 5°C temperature on *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in wheat grain

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Abstract

The effects of the insecticides chlorpyrifos-methyl and pirimiphos-methyl in combination with low temperature treatment at 5°C were tested in the laboratory to improve the existing pest management programs for *S. oryzae* control. Adults were released into wheat grain pretreated with three insecticide doses: 0.08, 0.12 and 0.16 mg/kg of chlorpyrifos-methyl, and 0.125, 0.19 and 0.25 mg/kg of pirimiphos-methyl, and exposed to 5°C temperature over intervals of 5, 6, 7 and 8 days. Mortality after low temperature only, insecticides only, and their