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June 20, 2023

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TO WHOM IT MAY CONCERN:

In Pistachio Day January 18, 2023, I presented a talk entitled Aflatoxin Management in Pistachio. At the end of this talk, I had a few slides where the sporulation of Afla-Guard vs. AF36 Prevail was compared under different relative humidities (laboratory tests). In that experiment, Afla-Guard GR spourulated much better than AF36 prevail in all, 91%, 96.5%, and 100% relative humidity. In a previous study again in the laboratory, Afla-Guard GR sporulated in higher rates than AF36 Prevail in the temperatures and the various soil moistures tested. The general conclusion was that undoubtedly, the Afla-Guard GR sporulated better even in lower temperature and in drier soil than the AF36 Prevail. I also reported on the predation of the two products in the field placed side by side. In this comparative study, both products showed good sporulation side by side, but within 24 hours , all the AF36 Prevail sorghum seeds were damaged, while the Afla-Guard seeds were left intact. It seems that ants/beetles have initial preference for damaging and/or removing AF36 Prevail sorghum seeds first; they do not touch (damage) the sporulating seeds of either product. In a third experiment, all the seeds of AF36 Prevail were removed by insects (ants and beetles) in 2 days after placing them in the field while the majority of the Afla-guard seeds were still in the place where they were placed. In another experiment where the seeds were placed in petri plates under direct sunlight, the Afla-Guard GR was more sensitive the sunlight preventing sporulation even after 1 day exposure in the direct sunlight while AF36 Prevail had some seeds (25%) sporulating even after 3 days exposure. As you can see there are more advantages of Afla-Guard GR in sporulation capacity with the exception of direct sunlight. All these experiments need to be repeated in order to make definite conclusions. And that is the reason this proposal was submitted to get support and hire a person to devoted all of his/her time to repeat this and other experiments.

Furthermore, additional tests of co-inoculation of toxigenic and the atoxigenic strains (AF36 and/or Afla-Guard) were performed in the laboratory with contradicting results. For instance, in these co-inoculation studies, we found that although AF36 prevented aflatoxin production by the toxigenic *Aspergillus flavus* strain, the Afla-Guard strain in one experiment reduced aflatoxin by only a small degree, while in another experiment increased the level of aflatoxin production by the toxigenic *A. flavus* in co-inoculated pistachio kernels. In another experiment, the Afla-Guard strain reduced aflatoxins produced by *A. parasiticus* more so than the AF36 strain. Getting this type of results suggests perhaps there was a chance of contamination of inoculum used to inoculate the pistachio

kernels in the first co-inoculation experiment. In commercial application at different times, Afla-Guard resulted in lower aflatoxin in the early application, while AF36 showed lower aflatoxin levels in the standard application time (see attached Xcel file).

Therefore, the submitted proposal, if funded, will give us the opportunity to resolve these issues by repeating experiments carefully with higher number of replicates to reach firmed conclusions.

In conclusion, both these registered products show advantages and disadvantages and should be used in the field, with no hesitation.