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**Science, Technology, Research and Innovation for Development
(STRIDE)**

PHASE I

Search for Non-aflatoxigenic *Aspergillus flavus* Isolates in Northern Philippines as Potential Biological Control Against Pre-harvest Aflatoxin Contamination of Peanut



PHASE II

Development of Protocols for the Production of Inoculants and Field Application of Atoxigenic Strains of *Aspergillus flavus* as Biological Control Agents Against Pre-harvest Aflatoxin Contamination of Peanut

- GRANTEE:** Benguet State University (BSU)
- PRINCIPAL INVESTIGATOR:** Dr. Janet Luis
- INDUSTRY PARTNER:** Lussoc Santo Domingo Ilocos Sur Farmers' Association
- US INDUSTRY PARTNER:** The University of Georgia–Tifton Campus
- GRANT PERIOD:** Phase I: PURE
Phase II: CARWIN
- GRANT AMOUNT:** May 1, 2015 to April 30, 2016
December 1, 2016 to November 30, 2017
- CONTRACT AMOUNT:** Php 8,969,756 (approximately USD199,300)

The thing about peanuts

Peanuts are an excellent source of cash for both small and large Filipino farms, which grew 29,000 tons of peanuts on 25,600 hectares in 2013. Peanut crops fit in nicely with current trends in organic farming and good agricultural practices, given peanut plants' ability to fix nitrogen. Peanuts are normally grown in a shortened maturity period, after rice, in the lowlands, with corn in Cagayan Valley, or in the uplands as a rain-fed crop. Peanuts are grown in a semi-arid environment that equally favors growth of aspergilli and aflatoxin contamination.



Collection of peanut samples by the research team, DA and farmers where the 9 ASAFs were isolated.

Peanuts, due to their high protein and oil content, are susceptible to *Aspergillus flavus* and *A. parasiticus* infection that produces aflatoxin. Aflatoxin is highly toxic and carcinogenic. The aspergilli prefer kernels with a post-harvest moisture content of 10.5-11% at 20-35°C and >83% relative humidity (CAST, 2003). When improperly

handled, processed peanut products can harbor aflatoxin beyond the acceptable limit of 20 parts per billion (ppb). This can happen in homemade products that do not undergo aflatoxin detection. In addition, pre-harvest aflatoxin contamination of peanuts is enhanced by the interrelation of prolonged drought and high soil temperature of 27-35°C during the last 3-6 weeks before harvest (Abbas, et al., 2009).

The phenomenon of biological control, or using mass-produced non-aflatoxigenic *A.flavus* strains to compete with field populations of aflatoxigenic *A.flavus* and *A. parasiticus* through pre-harvest treatment of peanuts with Afla-Guard in the US and Aflasafe in Africa, provides hope and a solution to the problem.

However, neither product can be imported or used in the Philippines given the environmental differences that affect activity of non-aflatoxigenic strains and the prevailing native aflatoxigenic fungi population. In Phase I, this project collected and isolated non-aflatoxigenic *A.flavus* strains with permanent molecular mutations from peanut-growing areas of the country to be developed as biological control agents like Afla-Guard and Aflasafe.



Isolation of non-aflatoxigenic A. flavus strains with permanent molecular mutations from peanut

Milestones

Phase I successfully identified four fungal strains lacking the entire aflatoxin biosynthetic gene cluster (ABGC), and five strains with partial defects in ABGC. These nine mutant atoxigenic strains of *A.flavus* (ASAFs) will form the basis of a biological control agent product with commercial potential that is effective against aflatoxin contamination in peanuts grown in the Philippines.

These significant findings led to a Phase II of the project. In Phase II, the project will evaluate appropriate protocols for the laboratory production of ASAF inoculants and field application of the four ASAFs with permanent mutation and five with partial mutation. The inoculants will become farmer-level biological control agents against pre-harvest aflatoxin contamination in peanuts. This protection will eventually extend to corn crops via the ASAFs Competitive Exclusion Technology. The project's Phase II will complete the needed protocols for the immediate commercialization of the product, proposed this early to be named AflaProtect.