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Research Application Summary

Effect of legume extracts on root rot pathogens and germination of common bean *(Phaseolus vulgaris)*

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Abstract

Application of undecomposed green manure have been reported to result in poor germination and crop establishment thus poor yields. To understand the mechanism through which low emergence occurs, various legume extracts from lablab, soybean, beans and groundnut were tested common bean germination and root rot pathogens by evaluating the extracts on germination of bean seed, mycelial growth, and spore germination. Observations were made on bean germination percentage, shoot length, dry weight of seedlings, and on mycelial growth, spore germination and number of germtubes per spore. The highest inhibition of bean germination was observed in aqueous and ethanol lablab extracts (60%) compared to the control with increased mean germination time (7 days) and corresponding decrease in germination index (2) and final germination percentage. Aqueous extract of lablab resulted in significantly high spore germination percentage (84%) with pronounced effect on germtube length (8%) and number of germtubes by 13% more than the control. Aqueous lablab extracts inhibit germination and stimulates germination of Fusarium spores that may be responsible for reduced germination and establishment of beans.

Keywords: Germination inhibition, mycelial growth, plant extracts, root rot, spore germination

Résumé

Il a été signalé que l'épandage d'engrais vert non décomposé entraîne une mauvaise germination et une mauvaise implantation des cultures, donc de faibles rendements. Pour comprendre le mécanisme par lequel une faible émergence se produit, divers extraits de légumineuses du lablab, du soja, des haricots et des arachides ont été testés sur la germination des haricots et les agents pathogènes de la pourriture des racines en évaluant les extraits sur la germination des graines de haricot, la croissance mycélienne et la germination des spores. Des observations ont été faites sur le pourcentage de germination des haricots, la longueur des pousses, le poids sec des semis, et sur la croissance mycélienne, la germination des spores et le nombre de tubes germinatifs par spore. L'inhibition la plus

élevée de la germination des haricots a été observée dans les extraits de lablab aqueux et éthanol (60%) par rapport au témoin avec une augmentation du temps de germination moyen (7 jours) et une diminution correspondante de l'indice de germination (2) et du pourcentage de germination final. L'extrait aqueux de lablab a entraîné un pourcentage de germination des spores significativement élevé (84%) avec un effet prononcé sur la longueur du tube germinal (8%) et le nombre de tubes germinatifs de 13% de plus que le témoin. Les extraits aqueux de lablab inhibent la germination et stimulent la germination des spores de Fusarium qui peuvent être responsables de la germination réduite et de l'établissement des haricots.

Mots-clés: inhibition de la germination, croissance mycélienne, extraits de plantes, pourriture des racines, germination des spores

Background

Common bean (*Phaseolus vulgaris*) is the most widely cultivated species of the Phaseolus genus (FAO, 2012). The crop forms significant part of diet in many households providing total protein (Wachenje, 2002) vitamins and micronutrients such as Zn and Fe (Welch *et al*., 2000). In spite of the nutritional and economic importance of beans, smallholder farmers encounter both abiotic and biotic constraints. Common bean seeds are associated with a range of fungi, however, root rot pathogens reflect negatively on growth and yield produced (El-Gali, 2015). Root rots caused by Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina, and Fusarium oxysporum heavily infect beans influencing growth from germination limiting the yield of bean crop (Naseri, 20008). Low soil fertility and low fertilizer use is a primary constraint to crop production throughout the developing world. The use of green manure as an alternative source for soil nutrient are widely documented (Mwangi and Mathenge, 2014). Green manure when applied to the soil increases soil carbon and organic matter and thus improves soil nutrient status (Talgre *et al*., 2009). Green manure enhances soil physical conditions by improving soil tilth, soil aeration, soil water holding capacity (WHC), plant root penetration, and suppression of pathogenic organisms, however, researchers have shown that incorporation of green manure many a times results in poor emergence and stand establishment. When plant residues decompose they releasing secondary metabolites that are phytotoxic to other plants (Kaur *et al*., 2012). However, Phytotoxicity depends on the amount of plant residues, the environment of decomposition, decomposition time, residue placement and weathering (Jilani *et al*., 2008). The phytotoxins found in plant materials inhibit growth and stimulate the population of root rot pathogens (Farooq *et al*., 2013). In view of the above outcomes, it is necessary to understand the cause of poor germination by utilizing extracts from legumes.

Study Description

Four legume plants common bean, lablab, soybean and groundnut were used in this study. The fresh legume residues were chopped into small pieces and macerated using a blender in sterile distilled water and in ethanol (80%) separately in the ratio of 1:10 (Cieniak *et al*., 2015). The mixture was left for 2 hours then pressed through layers of sterile cheese cloth and separated by filtration using Whatman no.1 filter paper (Whatman plc, Maidstone, Kent, UK). Solvent

(ethanol) was evaporated at lower temperature under reduced pressure in rotary flash evaporator to get the crude extracts. Fifty uniform bean seeds GLP2 variety were soaked in 200ml of different legume extracts overnight then sown in moist chamber lined with sterile paper towel. The chambers arranged in completely randomized design with four replicates were incubated and seeds allowed to germinate. Data on percent germination, germination index, MGT was collected. Shoot length was collected by selecting randomly 10 plants and measured.

On the activities of extracts on the mycelia and spore growth of *Fusarium solani*, two milliliters of each extract was added per Petri dish with 15ml of molten PDA and inoculated with 5mm of mycelial discs from 8 day old cultures and incubated at room temperature. Radial growth (mm) was measured after 72 up to 120 hours. Spores from Fusarium solani were harvested from 10 day old cultures by adding 5ml of sterile distilled water with tween 80 0.1% (v/v) to each petri dish and scrapping the surface using sterile glass slide. The spore suspension collected was centrifuged at 25^{O}C at 2000r/min for five minutes. The spores of strength 102 was prepared and using sterile micropipette, 50µl of spore suspension was drawn and mixed with each 50µl extract in a cavity of sterile slides (Amadi *et al*., 2014) and kept in moist chamber lined with sterile paper towel. All the treatments were arranged in Complete Randomized Design with four replications. Spore germination, number of germtubes per spore and germtube length was recorded in each of the four replicates after 24 hours.

Results

Effects of extracts on germination, emergence and mean emergence time of common beans. Bean seeds treated with aqueous legume extracts had high germination percentage when compared with ethanol extracts (Table 1). Ethanol extracts from lablab (41%), groundnut (48%) and aqueous lablab extracts (58%) had the lowest germination respectively while aqueous groundnut extracts (85%) had the highest germination percentage. Germination index was also affected by the legume extracts, the maximum value of germination index (GI) was recorded in seeds treated with sterile distilled water (control) followed by aqueous extracts from groundnut 4.9 while the lowest germination index of 2.3 was recorded in seeds treated with lablab ethanol extracts. Maximum bean germination time of 7.5 was recorded in seeds treated with ethanol lablab extracts while the least was recorded in seeds treated with water. Similar to germination, legume ethanol extracts had significant high effect ($p \leq 0.05$) on the seedling growth of common beans. The ethanol extracts showed inhibitory activities on the shoot.

Effect of legume extracts on mycelial and spore germination of *Fusarium* root rot. Mycelial growth of *Fusarium* treated with aqueous extracts from lablab had the largest diameter (54mm) while ethanol extracts from soybean had the least (20mm) after 96 hours (Table 2). Aqueous extract of lablab resulted in the highest germination percentage of spore (84%), longest germtube length (1.8um) and highest number of germtubes per spore and stimulated germination of spore by about 11% when compared to the control. However, fresh ethanol extracts from lablab, bean, soybean and groundnuts recorded the least germination percentage (2%) with the shortest germtube length (0.3um), the least number of germtubes per spore and nearly inhibited spore germination (97%).

Table 1. Effect of extracts effect on germination and growth related characteristics of common beans

Legume extracts	Germination (%)	Germination	Mean Germination (GI) Time (MGT)	Shoot length Index
Water				
Fresh Lablab	57.5_{c}	3.2_{c}	$4.4b_{c}$	5.9_{b}
Fresh Beans	72.5_{b}	4.1_{b}	3.9_{c}	4.6_{b}
Fresh Soybean	76.9_{b}	4.4_{ab}	4.9_{b}	13.2_{a}
Fresh groundnut	85.0_{b}	4.9_{a}	3.8_{c}	4.7_{b}
Alcohol				
Fresh Lablab	41.3_{d}	2.3_{d}	7.5_{a}	0.2_{d}
Fresh Beans	65.0_{bc}	3.7_{bc}	4.6^{b}	1.5_{c}
Fresh Soybean	76.3_{b}	4.4_{ab}	7.1^{a}	1.3_{c}
Fresh groundnut	48.1_{cd}	2.7_{d}	6.9^{a}	2.1_{c}
Control	99.4_{a}	5.7_{a}	3.0^{d}	12.6_{a}
Mean	62.6	3.6	5.3	4.6
LSD $^{(0.05)}$	12.4	0.7	0.9	2.4
CV (%)	13.9	13.9	12.8	36.7

Values followed by the same letter within the same row are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

Table 2. Colony diameter, spore germination, germtube length, number of germtubes per spore and germination inhibition of *Fusarium* under different legume extracts

Legume	Mycelial Growth (96 hours)	Spore germination (%)	Germtube length	germtubes per spore	(%) spore germination inhibition
Water					
Lablab	54.2_{a}	84.3_{a}	1.8_{a}	2.8_{ab}	-13.9_{c}
Beans	32.2_{b}	54.6_{a}	0.7b	1.9_{ab}	77.8_{a}
Soybean	44.4_{ab}	71.8_{a}	0.8_{abc}	2.8_{ab}	48.6_{abc}
Groundnut	46.7_{ab}	50.4_{a}	0.6_{bcd}	2.7_{ab}	63.9_{ab}
Alcohol					
Lablab	33.3_{b}	2.2_{b}	0.3_{cd}	0.2_{b}	98.6_{a}
Beans	38.9_{ab}	5.9_{b}	0.3_{cd}	0.4_{b}	95.8_{a}
Soybean	20.0_{b}	8.7_{b}	0.3_{cd}	0.8_{b}	94.4_{a}
Groundnut	36.7_{ab}	10.0_{b}	0.2_{d}	0.4_{b}	94.4_{a}
control	52.3_{a}	73.3_{a}	1.7_{ab}	$2.4a_{b}$	0.0_{c}
Mean	20.4	39.2	0.7	1.6	68.5
LSD $^{(0.05)}$	17.6 47.1	0.6	1.4	31.0	
CV (%)	54.5	72.3	54.6	54.2	27.3

Values followed by the same letter within the same row are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$).

Discussion

Lablab extracts had significant inhibition on bean seed germination and therefore increased mean germination time when compared with other legume extracts. The results also show that the extracts significantly reduced shoot and dry weight of bean seedlings. Early seedling growth is very sensitive to phytotoxins thus germination percentage is used to measure phytotoxic effects of substances (Kato-Noguchi and Islam, 2014). The delay in germination was more pronounced in seeds treated with ethanol extracts suggesting that inhibitory potential of the extracts is dependent on the extract medium used thus ethanol is more efficient in extracting bioactive compounds than water., however, the sensitivity of the extracts depended on the legume type. The results of the mycelium growth inhibition assay suggested that crude ethanol extracts from Lablab and soybean were the most active against Fusarium, however, aqueous crude extracts from lablab stimulated mycelial growth and germination of spores. Aqueous lablab extracts are stimulatory to root rot pathogens, therefore, when crops are planted immediately after incorporation, explosion of pathogenic microbes compounded by the release of phytotoxic compounds (Bonanomi *et al*., 2006) that damage plant roots and predispose them to attack by root rot pathogens (Ye *et al*., 2004). The inhibition is as a result of phytotoxins released by a crop during decaying. Fresh lablab residue extracts negatively affect common bean germination, enhance spore germination and mycelial growth and thus increases the population of Fusarium root rot pathogens. The effect of these substances is the injury they impart on the crops that results in reduced and delayed germination.

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References

Ayub, M., Ijaz, M. K., Tariq, M., Tahir, M. and Nadeem, M. A. 2012. Allelopathic effects of winter legumes on germination and seedling indicators of various summer cereals. *Agricultura Tropica et Subtropica* 45: 179-183.

Bonanomi, G., Sicurezza, M.G., Caporaso, S., Assunta, E. and Mazzoleni, S. 2006b. Phytotoxicity dynamics of decaying plant materials. *New Phytologist* 169: 571-578.

Cieniak, C., Walshe-Roussel, B., Liu, R., Muhammad, A., Saleem, A., Haddad, P.S., Cuerrier, A., Foster, B.C. and Arnason, J.T. 2015. Phytochemical comparison of the water and ethanol leaf extracts of the Cree medicinal plant, *Sarracenia purpure*a L. (Sarraceniaceae). *Journal of Pharmacy and Pharmaceutical Sciences*18:484-493.

El-Gali, Z.I. 2015. Influence of Seeds and Roots Extracts and Exudates of Bean Plant on Growth of Some Pathogenic Fungi. *Open Access Library Journa*l 2: e1666. http://dx.doi.

org/10.4236/oalib.1101666.

Jilani, G., Mahmood, S., Chaudhry, A. N., Hassan, I. and Akram, M. 2008. Allelochemicals: sources, toxicity and microbial transformation in soil - a review. *Annals of Microbiology* 58 (3): 351-357.

Kato-Noguchi, H., Mominul, A. K. M. and Islam. 2014. Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. *The Scientific World Journal* pp.1-8. http://dx.doi.org/10.1155/2014/676242.

Kaur, S., Singh, H. P., Batish, D. R. and Kohli, R. K. 2012. Phytotoxicity of decomposing below-ground residues of Ageratum conyzoides: nature and dynamics of release of phytotoxins. *Acta Physiologiae Plantarum* 34: 1075-1081.

Mohammedi, Z. and Atick, F. 2011. Impact of solvent extraction type on total phenols content and biological activity from *Tamarix aphylla* (L.) Karst. *International Journal of Pharma and Biosciences* 2: 609 – 615.

Mwangi, P. M. and Mathenge, P. W. 2014. Comparison of tithonia (Tithonia diversifolia) green manure, poultry manure and inorganic sources of nitrogen in the growth of kales (Brassicae oleraceae) in Nyeri county, Kenya. A*frican Journal of Food, Agriculture, Nutrition and Development* 14 (3): 8791 – 8808.

Naseri, B. 2008. Root rot of common bean in Zanjan, Iran: major pathogens and yield loss estimates. *Australasian Plant Pathology* 37:546-551.