https://doi.org/10.56093/ijas.v90i3.101472

Indian Journal of Agricultural Sciences **90** (3): 546–50, March 2020/Article

Method to prolong shelf-life of potato (*Solanum tuberosum*) micro-tubers under refrigerated condition

G RAVICHANDRAN, E P VENKATASALAM*, AARTI BAIRWA and R K SINGH

ICAR-Central Potato Research Station, Muthorai, Udhagamandalam, Tamil Nadu 643 004, India

Received: 06 March 2019; Accepted: 09 August 2019

ABSTRACT

The experiment was conducted at ICAR-Central Potato Research Station, Muthorai, Udhagamandalam, The Nilgiris during 2013–16 to test the effect of different bio-polymer coating materials on potato (*Solanum tuberosum* L.) micro-tubers for improving the storage life under refrigerated conditions. Potato micro-tubers (Kufri Swarna) produced through tissue culture technique were given coating with three different bio-polymers (chitosan, gelatin and chitin) in four different concentrations with three different coatings (single, double and triple layered) and stored under refrigerator (at 4°C) to see the effect of bio-polymers on shelf life of micro-tubers using completely randomized design (CRD). Among the chemicals and their concentrations tried, gelatin at 0.25% concentration with single layer coating was found effective in reducing the storage losses of micro-tubers considerably even up to 165 days storage. This treatment was also found effective in maintaining better stand establishment (58.97%) in the field with significantly higher yield (1.57 kg/m^2) than the control and other treatments. Hence, gelatin can be used for coating of micro-tubers in order to enhance the shelf life up to a period of 165 days under refrigerated conditions.

Key words: Bio-polymers, Chitin, Chitosan, Gelatin, *Solanum,* Shelf life protectant

The use of tissue culture technique in multiplication of disease free plants is rapidly widening because of faster multiplication rate. In potato (*Solanum tuberosum* L.) micro-tuber based seed production is one of the approaches used for the multiplication of disease free seed material. Production of micro-tubers in different potato growing areas is not feasible as it is a costly affair. Hence, the micro-tubers produced at one place needs to be transported to various seed producing areas, which need sufficient storage life for proper establishment and multiplication in the field. The micro-tubers are produced in liquid growth medium almost at 100% RH throughout their development therefore it cannot be stored for longer and tend to be very soft with open lenticels (Sharma *et al.* 2012). Hence, closing of these lenticels through a coating over micro-tubers using some bio-degradable polymers may provide a solution for the above problem. The present work was undertaken using bio-polymers as coating materials, which are safe and more eco-friendly. Chitosan is composed of β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit), which is well demonstrated as seed coating material in several studies (Goosen Mattheus 1997) and it had shown multiple benefits on seeds. It acted as antifungal agent in few crops (Bhaskara Reddy *et al.* 1999) through secretion of phenolic and phytoalexin substances (Martinez 2002) to reduce the microbial growth. Chitin is another variant of chitosan obtained from the same source but deviate in the manufacturing process, which is a macromolecule used for making artificial skins and sutures. Gelatin, a highly water soluble protein obtained from animal bones on refinement which is widely used in foods and pharmaceuticals for coating and micro-encapsulation (GMIA 2012) and also as a bio-herbicide formulation (Zhang *et al.* 2003). In this backdrop, a study was undertaken with coating of micro-tubers in different bio-polymers to minimize the water loss in order to increase the storage life.

MATERIALS AND METHODS

The experiment was conducted at ICAR-Central Potato Research Station, Muthorai, Udhagamandalam, The Nilgiris, Tamil Nadu located at an altitude of 2140 m amsl at 11°24' N latitude and 74°4' E longitude during the year 2013–16. The micro-tubers were produced at ICAR-Central Potato Research Institute, Shimla, India and air transported to the experimental station. The micro-tubers were stored in refrigerator at 4°C after giving the treatments up to 195 days.

Micro-tuber production: Shoot cultures of potato were produced using 13 ml of MS (Murashige and Skoog 1962) solid media in culture tubes (150 × 25mm). Cultures were kept under 16 h photoperiod of 3000-4000 lux with a temperature of 22 ± 1°C, for 25-28 days until shoots were grown to full height of culture tubes. Micro-tuberization was carried out as per the procedure described by Naik and

*Corresponding author e-mail: venkat_ep@yahoo.co.in

Sarkar (1997). Thus produced micro-tubers were allowed to become green at 16 h photoperiod at 22 ± 1°C for a week. Micro-tubers of 8 mm size after size grading were used for this experiment.

Treatments: The micro-tubers of potato were treated with three bio-polymer materials (gelatin, chitosan and chitin) in four concentrations (0.25, 0.50, 0.75 and 1.00%) and each concentration was tried with three types of coating, viz. single (S_1), double (S_2) and triple (S_3) layered coating. Distilled water (single distillation) was used as control with four levels of water coating (Total tuber immersing volume was calculated and is taken as T_1, double the water volume is T_2, triple the volume is T_3 and four times the volume is T_4. In total there were 48 treatments with three replications. Three kinds of bio-polymers, viz. chitosan, gelatin and chitin were procured from Biogen (USA). Acetic acid with 99.7% purity and hydroxyl sodium solution (1N) were procured from Merck (India) and used for dissolving the chitosan and chitin respectively. Different concentration of chitosan solution, viz. 0.25, 0.50, 0.75 and 1.00% was prepared by dissolving required quantity of chitosan powder in 10, 15, 20 and 25ml (1% w/w acetic acid) respectively. Whereas, different concentration of chitin solution, viz. 0.25, 0.50, 0.75 and 1.00% was prepared by dissolving required quantity of chitin powder in 5 ml HCL for 0.25 and 0.50% and in 7.5 ml HCL for 0.75 and 1.00%. It took 4 h for complete dissolving of chitosan 0.25 and 0.50% whereas, it was 6 h for 0.75 and 1.0%. However, different concentration of gelatin, viz. 0.25, 0.50, 0.75 and 1.00% was obtained by dissolving required quantity of gelatin in distilled water. After complete dissolving the final volume was made up to 100 ml with distilled water.

Coating solutions: Four concentrations of solutions were prepared for seed coating in all the polymers. Chitosan powder was dissolved as per the methodology described by Khalid *et al.* (2010) with some modifications (Table 1). Gelatin powder weighing 0.25, 0.50, 0.75 and 1.00 g was dissolved in 100 ml of distilled water to get respective concentrations for T_1, T_2, T_3 and T_4. Chitin was dissolved in concentrated hydrochloric acid (HCl) and then the *p*H was adjusted to 7.0 (Table 2). Distilled water was used as control.

Coating process: In all the treatments, a total of 15 tubers were selected and taken in to petridishes and 5ml of respective solution was added and then the petridishes were rotated gently by hand to get uniform coating. To eliminate the excess solution, the micro-tubers were dried using conventional hand dryer. For multilayer treatment the action was repeated two or three times. All the treatments were replicated thrice. S_1: Single time coating, S_2: Two times coating S_3: Three times coating

Coating thickness: The thickness of coating by polymers (thickness of the film) was measured by image analyzer using the software Prog Res® Capture pro, Version 2.7 x WIN.

Weight loss: During the storage, tuber weight loss was estimated at a regular interval of 15 days from 60 days onwards after the treatment till 165 days and it is expressed in percentage. But the weight loss was estimated up to 195 days. After 165 days, other parameters like sprout length

Table 1 Weight loss, sprout length and thickness of micro-tubers after 165 days of storage

Treatment	Weight loss (%)				Sprout length (mm)				Sprout thickness (mm)			
	Number of coating			Mean	Number of coating			Mean	Number of coating			Mean
	$S_{1\text{-single}}$	$S_{2\text{-double}}$	$S_{3\text{-triple}}$		$S_{1\text{-single}}$	$S_{2\text{-double}}$	$S_{3\text{-triple}}$		$S_{1\text{-single}}$	$S_{2\text{-double}}$	$S_{3\text{-triple}}$	
T_1-Chitin 0.25%	20.22	17.67	17.66	18.52^{B}	3.42	3.38	3.44	3.41^{I}	0.91	1.12	1.10	1.04^{CDE}
T_2-Chitin 0.50%	21.12	16.81	16.79	18.24^{B}	4.21	3.99	4.08	4.09^{GH}	0.72	0.98	0.80	0.83^{F}
T_3-Chitin 0.75%	19.82	16.82	16.79	17.81^{B}	3.62	3.49	3.57	3.56^{HI}	0.41	0.45	0.42	0.43^{G}
T_4-Chitin 1.00%	13.68	16.50	10.73	13.64^{C}	4.54	4.72	4.64	4.63^{G}	0.42	0.41	0.42	0.42^{G}
T_5-Gelatin 0.25%	19.28	17.30	17.20	17.93^{B}	6.22	6.27	6.12	6.20^{F}	1.95	1.22	1.21	1.46^{A}
T_6-Gelatin 0.50%	19.13	16.90	16.91	17.64^{B}	6.28	6.48	6.37	6.38^{EF}	1.32	1.31	1.33	1.32^{B}
T_7-Gelatin 0.75%	19.83	16.98	16.93	17.91^{B}	6.89	6.71	6.91	6.84^{DE}	1.31	1.28	1.29	1.29^{B}
T_8-Gelatin 1.00%	19.96	17.71	17.74	18.47^{B}	8.11	7.99	8.05	8.05^{BC}	1.10	1.14	1.13	1.12^{C}
T_9-Chitosan 0.25%	19.81	17.22	17.70	18.24^{B}	7.16	7.22	7.06	7.14^{D}	1.08	1.05	1.04	1.06^{DE}
T_{10}-Chitosan 0.50%	19.22	17.11	17.42	17.92^{B}	7.49	7.42	8.51	7.81^{C}	1.03	0.99	1.03	1.02^{DE}
T_{11}-Chitosan 0.75%	19.89	17.34	17.51	18.23^{B}	8.42	8.55	8.45	8.47^{AB}	1.01	1.21	1.11	1.11^{CD}
T_{12}-Chitosan 1.00%	19.91	15.10	15.02	16.68^{B}	8.62	8.66	8.95	8.74^{A}	1.00	1.00	1.01	1.00^{E}
T_{13}-Control single	22.25	23.35	22.65	22.75^{A}	3.44	2.94	3.88	3.42^{I}	0.45	0.48	0.39	0.44^{G}
T_{14}-Control double	23.32	23.22	23.15	23.23^{A}	3.29	2.99	3.58	3.29^{I}	0.47	0.51	0.37	0.45^{G}
T_{15}-Control triple	24.85	24.43	22.67	23.98^{A}	3.19	3.23	3.29	3.2^{I}	0.39	0.46	0.39	0.41^{G}
T_{16}-Control quadruple	25.26	23.94	23.43	24.21^{A}	3.25	3.19	3.45	4.00^{I}	0.44	0.39	0.41	0.41^{G}

Table 2 Effect of polymer coating on per cent establishment, plant characters and yield parameters

Treatment	Establishment (%)*	75 days after planting			Per m^2	
		Plant height	No. of Stems per plant	No. of leaves per plant	No. of tubers	Yield (kg)
T_1-Chitin 0.25%	31.95	78.00	1.00	18.13	21.33	1.04
T_2-Chitin 0.50%	26.09	0.00	0.00	0.00	0.00	0.00
T_3-Chitin 0.75%	28.30	0.00	0.00	0.00	0.00	0.00
T_4-Chitin 1.00%	28.82	0.00	0.00	0.00	0.00	0.00
T_5-Gelatin 0.25%	58.97	91.00	1.67	29.18	28.00	1.86
T_6-Gelatin 0.50%	56.71	95.67	1.17	23.80	34.33	1.63
T_7-Gelatin 0.75%	32.05	85.00	1.33	21.82	25.67	1.40
T_8-Gelatin 1.00%	60.77	93.33	1.00	25.50	30.00	1.38
T_9-Chitosan 0.25%	57.55	65.33	1.00	16.43	19.33	1.18
T_{10}-Chitosan 0.50%	70.20	75.33	1.00	18.98	22.33	1.13
T_{11}-Chitosan 0.75%	65.94	85.00	1.00	22.38	26.33	1.15
T_{12}-Chitosan 1.00%	60.77	86.00	1.00	22.10	26.00	1.04
T_{13}-Water single	46.92	40.33	1.00	9.35	11.00	0.69
T_{14}- Water double	50.95	45.33	1.00	10.48	12.33	0.70
T_{15}- Water triple	47.31	47.33	1.00	11.05	13.00	0.68
T_{16}- Water quadruple	48.36	51.00	1.00	11.90	14.00	0.62
CD (P=0.05)	6.96	6.66	0.15	2.81	3.24	0.17

*Establishment % values are arcsine transformed

and thickness were not able to recorded, weight loss of the micro-tubers has not been presented here.

Sprout length and thickness: Sprout length and thickness were measured in 5 tubers in three replications (total 15 tubers) at random with the help of Image analyzer using the software Prog Res® Capture pro. Length measurements were taken from the point of sprout formation up to the end of the sprout. Method followed for the measurement is illustrated in Fig 1. Sprout thickness was measured in three places leaving 0.25 ± 0.006 mm in both the sides, averaged and expressed in mm. Sprout length and thickness

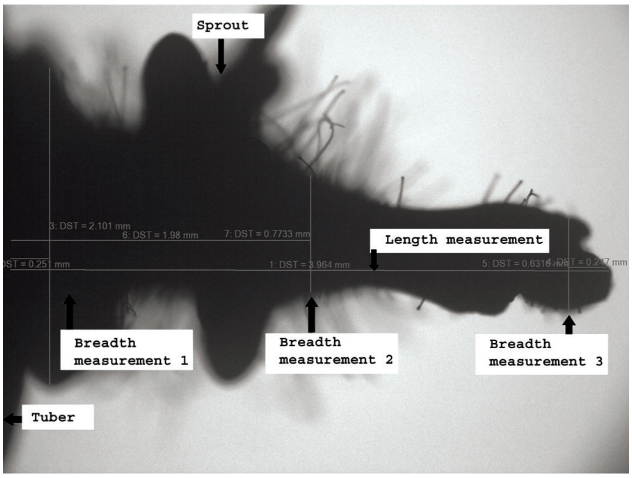

Fig 1 Procedure followed in the sprout length and thickness measurement using image analyser (Vertical line indicates the length and horizontal lines indicate the thickness).

was measured at a regular interval of 15 days from 60 days onwards till 165 days of storage only.

Planting: Planting of treated and stored micro-tubers was done inside the net house with a spacing of 50 × 20 cm. Tubers were placed manually at a depth of 5±1 cm. Recommended cultural practices were followed. Stand establishment percentage and yield data were recorded. The establishment was very poor and erratic in 180 and 195 days stored tubers. Hence observations were restricted only to 165 days stored tubers.

Experimental design and statistical analysis: In vitro experiments were conducted using Completely Randomized Design (CRD), whereas, *in vivo* experiment was conducted in a factorial completely randomized design. Data were analyzed statistically by applying the technique of analysis of variance (ANOVA) as described by Gomez and Gomez (1984). Mean values were calculated and separated using Tukey Significant Difference test at the 5% significance level.

RESULTS AND DISCUSSION

Coating thickness: Using image analyzer the coating thickness over the micro-tuber skin was measured and all the three bio-polymers formed a coating thickness of 10 to 420 µm, the coating thickness varied due to coatings but not by concentrations. The chitosan and chitin formed a layer like structure whereas, gelatin formed granular structure all over the tubers.

Behaviour of coated micro-tubers during storage

Tuber weight loss: Weight loss of tuber was significantly influenced by treatments and the number of coatings however, there was no significant interaction effect. Different concentration of polymer coating significantly reduced the weight loss over control from 60 days of storage. At 165 days, micro-tubers soaked in different volume of water (T_{13}-T_{16}) recorded the highest weight loss of 22.75–24.21% and the polymer coated tubers were able to manage reduced weight loss to the tune of 13.64–18.52% (Table 1). In stored potatoes, loss of weight occurs mainly due to two physiological processes namely transpiration and respiration. The reduced water loss in storage due to the application of polymer coating might be due to reduced water evaporation from micro-tubers as chitosan is having film making character to retain water content (Meyers *et al.* 2008), whereas, gelatin could form thermo reversible gels. Chitosan and chitin contains calcium, which might have played a role in stopping the loss of cell integrity, ion transport regulation and thereby water loss (Saad and Mostafa 2012).

Sprout length and thickness: The sprout length was significantly affected only by treatments. In general two of the polymers chitosan and gelatin enhanced the sprout length in comparison to control. Among different concentration of polymers chitosan (0.75–1.00%) and gelatin (1.00%) resulted production of longer sprouts (Table 1).

Sprout growth in potato is an indication of release of dormancy and start of plant growth. Short or rudimentary sprout is not fit for giving out a good plant, due to lack of necessary energy, vitamins, minerals, protein and all needed desirable nutritional changes (Chavan *et al.* 1989). As micro-tubers are miniature structure of potato tubers, the nutritional capacity to give a better sprout is remote and it needs an exogenous application. Control of sprouting is critical for potato storage to maintain its firmness, weight and texture (Source *et al.* 2005).

In this study, the sprout thickness was significantly affected by different treatments and interaction of different treatments with number of coatings. In general micro-tubers treated with different concentration of gelatin recorded the thicker sprouts, followed by chitosan treated ones as compared to chitin and water treated control (Table 1). In potato, the sprout performance is judged through the thickness and length. Sprout thickness is also important for potato seed tubers for further better performance in the field. The nutrients available to the bud along with environment may have a role in the sprout thickness. Gelatin has got many amino acids, such as, glycine, proline, hydroxyproline, glutamic acid etc. which might have played a vital role in improving the sprout thickness by providing better nutrition.

Performance of micro-tubers in net house: As there was no significant difference between the single and multiple layers of coating of polymers on micro-tuber sprout growth. Therefore, only single coated micro-tubers were tested for its performance under net house condition.

Stand establishment: Chitosan and gelatin treated micro-tubers resulted better establishment than control whereas, chitin treatment had negative effect on establishment. Gelatin with sturdy sprouts could have established well soon after planting and that is the reason for its better establishment. Chitosan could maintain better establishment due to its anti fungal and moisture retaining properties as well as good sized sprouts. Though, there was an establishment in chitin treated tubers but barring 0.25% concentration, no plant survived in other concentrations after its establishment. This could be correlated with its abysmal performance during storage where it could produce only very thin and lanky sprouts (Table 1).

Plant growth and yield parameters: Significant differences were observed in all the plant growth parameters due to bio-polymer coatings. Different concentration of gelatin coated micro-tubers performed well with respect to plant height at 60 days, number of stems at 75 days and number of leaves both at 60 and 75 days after planting in the net house. Among the concentrations, 0.25% gelatin increased number of stems and leaves per plant as well as plant height (Table 2). This was closely followed by chitosan treated tubers which were also significantly superior to chitin and control. Micro-tubers treated with Chitin performed very poor and it had shown some negative effect both on growth characters and yield parameters.

Potato tuber is considered as balanced and nutritionally favorable but the essential aminoacids methionine and cysteine are underrepresented (Burten 1989). Galili and Hofgen (2002) reported that the seeds accumulate amino acids to be used later as sources of nitrogen and energy for further growth. Hence, the amino acids supplied through gelatin treatment might have given a support for the better performance in the net house. A better and healthy crop can have a good source sink movement which results in the bulking of tubers. Gelatin and chitosan at all concentrations produced significantly superior yield over control in terms of tuber number as well as weight.

To study the relationships between various factors which determine the storability of micro-tubers and the field performance in terms of growth characters and yield parameters, Pearson's correlation coefficients were estimated between these parameters. Tuber yield of the net house planted crop was positively correlated with sprout thickness, tuber number, plant height, number of stems and number of leaves, but, negatively correlated with sprout length and tuber weight loss in storage. Morris (1966) stated that the rate of sprout growth was inversely related to the number of competing sprouts, length of sprouts tended to increase with an increase in the weight of the mini-tubers. Further, mother tuber size was positively correlated with mean sprout length (Table 3). Among the different growth and yield parameters, tuber number, leaf number and plant height have shown a very high degree of correlation with that of tuber yield. This indicates that the treatment which can give more thick and sturdy stems will also perform better in the net house with better vigour and the same will also perform better in terms of mini-tuber productivity.

The shelf life, viability and net house performance of microtubers was increased by single layer coating of

Table 3 Relationships between various growth and yield parameters as influenced by polymer coating

	Weight loss (%)	Sprout. Length	Sprout thickness	No. of stems	No. of leaves	Tuber number	Tuber yield
Weight loss (%)	1.000	-0.620	-0.620	-0.167	-0.136	-0.132	-0.116
Sprout length	-0.620	1.000	0.702	-0.441	-0.202	-0.204	-0.200
Sprout thickness	-0.620	0.702	1.000	0.054	0.204	0.201	0.332
Plant height	-0.106	-0.271	0.139	0.817	0.974	0.974	0.915
No. of stems	-0.167	-0.441	0.054	1.000	0.747	0.748	0.827
No. of leaves	-0.136	-0.202	0.204	0.747	1.000	1.000	0.934
Tuber number	-0.132	-0.204	0.201	0.748	1.000	1.000	0.935
Tuber yield	-0.116	-0.200	0.332	0.827	0.934	0.935	1.000

Gelatin (0.25%). Further, in comparison with chitosan, gelatin is easily available and can be easily dissolved in water. Hence, gelatin is recommended to be used as shelf life protectant of potato microtubers up to a period of 165 days after harvesting under refrigerated conditions.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge Dr K P Chandran, Principal Scientist (Statistics), ICAR-Central Plantation Crops Research Institute, Kasaragod, for help in compiling and analysing the data and Director, ICAR-CPRI, Shimla for providing facilities and funds.

REFERENCES

Bhaskara Reddy M V, Arul J, Angers P and Couture L. 1999. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. *Journal of Agriculture & Food Chemistry* **47**(3): 1208–16.

Burten W G. 1989. The nutritive value of potatoes, cooked or processed, in relation to human requirement. *The Potato,* pp. 365–90. Burten W G (Eds). Longman, UK.

Chavan J K, Kadan S S and Larry R B. 1989. Nutritional Improvement of cereals by sprouting. *Critical Review in Food Science and Nutrition* **28**(5): 401–37.

Galili G and Hofgen R. 2002. Metabolic Engineering of Amino acids and Storage Proteins in plants. *Metabolic Engineering* **4**: 3–11.

GMIA. 2012. *Gelatin Handbook*, pp 7. Gelatin manufactures Institute of America.

Gomez K A and Gomez A A.1984. *Statistical Procedures for Agricultural Research*. John Wiley & Sons, New York.

Goosen Mattheus F A.1997. *Applications of Chitin and Chitosan*. CRC Press LLC, Boca Raton, Florida.

Khalid Z, Beatriz U and Juan I M. 2010. Application of bio active coatings based on Chitosan for artichoke seed production. *Crop Protection* **29**: 853–59.

Leclerc Y, Donnelly D J and Seabrook J E. 1994. Microtuberization of layered shoots and nodal cuttings of potato: the influence of growth regulators and incubation periods. *Plant Cell Tissue and Organ culture* **37**: 113–20.

Naik P S and Sarkar D.1997. Influence of light induced greening on storage of potato microtubers. *Biologica Plantarum* **39**: 31–4.

Martinez P A. 2002. Biological pesticide based on chitosan and entomopathogenic nematodes. Patentee: WO/2002/037966.

Meyers M A, Lin A Y M, Seki Y, Chen P Y, Kad B K and Bodde S. 2008. Biological Materials: Structure and Mechanical Properties. *Progress in Material Science* **53**: 1–208

Morris D A. 1966. Inter sprout competition in the potato I. Effects of tuber size, sprout number and temperature on sprout growth during storage. *Potato Research* **9**: 69–85.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiology of Plant* **15**(3): 473–97.

Saad M H and Mostafa M R. 2012. Effects of calcium paste as a seed coat on growth, yield and enzymatic activities in NaCl stressed-pea plants. *African Journal of Biotechnology* **11**(77): 14140–5.

Sharma A K, Venkatasalam E P and Kumar V. 2012. Storability and sprouting behavior of micro-tubers of some Indian potato cultivars. *Potato Journal* **39** (1): 31–8.

Source C, Lorenzi R, Parisi B and Ranalli P. 2005. Physiological Mechanisms Involved in Potato (*Solanum tuberosum*) Tuber Dormancy and the Control of Sprouting by Chemical Suppressants. *Acta Horticulture* **684**: 177–86

Zhang W, Wolf T M, Bailey K L, Mortensen K and Boyetchko S M. 2003. Screening of adjuvants for bioherbicide formulations with *Colletotrichum* spp. and *Phoma* spp. *Biological Control* **26**: 95–108.