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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (5): 420-426, 2009
ISSN 1028-8880

A Comparative Study Between Detached Leaf and Plastic Growth Pouches Techniques on the Infection of Pseudocercospora Leaf Spot Disease of Cowpea Cultivars (*Vigna unguiculata* L. Walp) in Northeast Thailand

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Abstract: This glasshouse experiment was carried out in the 2004 at the Department of Agricultural Production, Faculty of Technology, Mahasarakham University, Mahasarakham 4400, Northeast Thailand to compare amounts of infection of Pseudocercospora leaf spot disease in leaves of cowpea cultivars being screened between detached leaf and plastic growth pouches techniques. The experiment was laid in a factorial arranged in a Randomized Complete Block Design (RCBD) with four replications. KVC7, IT81D-1228-14-1 and KKU25 cowpea cultivars were used as factor A, detached leaf and plastic growth pouches techniques were used as factor B and four levels of the concentrations of the spores of the disease, i.e., 0, 1×10^4, 5×10^4 and 1×10^5 of disease forming units mL^{-1} of distilled water were used as factor C. The results showed that KVC7 and IT81D-1228-14-1 cultivars possessed immune and high resistant properties against the disease, respectively, whereas KKU25 cultivar ranked as a susceptible cultivar. Detached leaf technique gave higher amount of infection of the disease than plastic growth pouches technique and the differences were highly significant. A plentiful amount of I nfected disease was attained with an inoculation rate of 1×10^5 forming units mL^{-1} of distilled water, which was considered too high and the most appropriate concentrations for use lie on both concentrations, i.e., 1×10^4 and 5×10^4 forming units mL^{-1} of distilled water. An interaction among factors A×B×C was severely found with KKU25 cultivar, particularly on day 28 after inoculation, whilst other two cultivars gave a minute amount of disease incidences (%) where detached leaf technique gave much higher disease incidences than plastic growth pouches technique, thus detached leaf technique should be of higher advantages.

Key words: Concentration of spores, cowpea cultivars, detached leaf technique, disease, glasshouse conditions, inoculation, plastic growth pouches technique

INTRODUCTION

It has been commonly practiced among plant breeders that some preliminary screenings for outstanding crop cultivars on disease infection are obviously carried out under glasshouse conditions due to some significant advantages, e.g., low cost of investment, a large number of sampling populations could be increased and environmental conditions could be controlled to attain a similar growth environments as that of natural conditions where the crop plants are normally grown. For example, many crop plants are traditionally grown in the rainy season but it is so happened that an investigation must be taken out in the dry season then a similar environmental condition to that of the rainy season must be established for the work, i.e., a glasshouse where control environmental conditions fitted to do the work must be available. It has been advocated that detached leaf technique could be one of many techniques being used for an inspection of any infected disease under glasshouse conditions when Melouk and Banks (1978) who was the first scientist to have carried out experiment in England with the use of detached leaf technique where the petioles of the detached leaves immerged in a Hoagland's solution with an aim to search for a high resistant property in groundnut plant (*Arachis hypogaea*) against Cercospora leaf spot disease. Melouk and Banks (1978) reported that the technique being used was successfully achieved when the inoculated disease was allowed to have its effect within a few weeks after

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allowing the detached leaves of the crop continue its biotic activities under the glasshouse conditions for a certain period of time. The study of Melouk and Banks (1978) with the use of the third and forth leaves (counted from top) of groundnut plants aiming to determine the infection of *Phaeoisariopsis personata* pathogenic disease in leaves but in place of nutrient solution they used sand particles as a medium covered with plastic sheets to avoid a rapid evapo-transpiration in leaves and the medium being used where they added distilled water daily to maintain adequate amount of moisture contents in the media. They reported that the results attained from the experiment were successfully examined at 30 days after a spraying of spores of the disease to leaves at a rate of 2×10^4 spores L^{-1}. Thus the technique being used was fairly acceptable where it economized the budget and time saving. Green and Wynne (1986) compared the results of the experiments between detached leaf technique carried out under glasshouse conditions and field experiment with the use of peanut plants on the disease known as early leaf spot. They concluded that the results attained under the glasshouse of the detached leaf technique were comparable to field experiment where both experiments gave a similar amount of the infection of the tested disease. With the study on cowpea (*Vigna unguiculata* L. Walp) of Sinsir (2006), they reported that the results derived from detached leaf technique carried out under glasshouse conditions gave a similar result on the infection of the disease as found with the results carried out under field conditions.

The use of plastic growth pouches for the determination of plant rhizobium had been carried out when Summerfield *et al.* (1977) used a modified Long Ashton growth medium to grow leguminous crops in plastic growth pouches and they reported that the results attained from the use of plastic growth pouches were successfully achieved. The use of plastic growth pouches was also carried out with rice plant experiment (*Oryza stativa*) when rice plants were allowed to grow in the aircraft in the space where scientists aimed to determine the infection of the disease of *Xanthomonas oryzae pv. oryzae* in rice plants and they were able to attain a successful work in the space (Chambers *et al.*, 1998). Furthermore, the use of plastic growth pouches on the selection of cowpea cultivars was successfully attained with the study of Francois *et al.* (2004). Therefore, the technique in growing plants with the use of plastic growth pouches has been widely accepted by a number of scientists. This study aimed to compare the effectiveness of the applied techniques, i.e., both detached leaf and plastic growth pouches on the selection for high resistant properties of cowpea cultivars against Pseudocercospora leaf spot disease if the attained results of both techniques could be comparable and which of them could provide the most appropriate advantages.

MATERIALS AND METHODS

The experiment was conducted under glasshouse conditions at Mahasarakham University Experimental Farm, Mahasarakham Province, Northeast Thailand in the rainy season of the 2004 to compare the results derive from both detached leaf and plastic growth pouches techniques on the infection of Cercospora leaf spot disease of cowpea cultivars. The experiment was laid in a factorial arranged in a Randomized Complete Block Design (RCBD) with four replications. Three cowpea cultivars, i.e., KVC7, IT81D-1228-14-1 and KKU25 were used as factor A, detached leaf and plastic growth pouches techniques were used as factor B and four levels of the spore concentrations of the disease, i.e., 0, 1×10^4, 5×10^4 and 1×10^5 of disease forming units mL^{-1} of distilled water were used as factor C. With the results of the earlier study, it was found that cowpea cultivars of KKU25, IT81D-1228-14-1 and KKU25 were ranking according to their performances against the disease as: susceptible, resistant and immune, respectively (Sinsiri *et al.*, 2006).

For the detached leaf technique, the method of Sinsiri and Laohasiriwong (2007) was used, i.e., 100 seeds of each cowpea cultivars were sown separately into three plots with a distance between rows and within rows of 20×15 cm, respectively. To avoid the contamination of the disease, the three plots were allocated in a glasshouse. Twenty six days after emergence, 2 fully expended adjacent leaves of the tri-foliage type of leaves of each plant sample were pulled off leaving only a single leaf for use in the detached leaf technique. The reason for pulling off of the two leaves of each plant away was to avoid a high evapo-transpiration rate in leaves of the ones being left out for the experiment. At day 28 after emergence, the prepared leaf of each plant was removed with a 5 cm long for each petiole of leaves and then submersed the petioles of leaves into an Indole-3-Acetic Acid (IAA) solution for 2 min (the solution has a concentration of 500 mg L^{-1} of distilled water). Some small amounts of IAA being absorbed by each leaf could possibly encourage root formation of each petiole when planted in growth media in plastic containers. The plastic containers of the same sizes (width and length) were used, i.e., each has a diameter of 7.5 cm with a lengthy dimension of 11.5 cm.

Each of the plastic containers has a drainage hole of approximately 0.50 cm in diameter and each contained sterilized sand particles of approximately 90% of its full capacity and then they were placed in a plastic tray filled with distilled water at a level of approximately 3-4 cm, where, the plastic containers could attain a certain amount of distilled water by capillary absorption. After allowing the sand particles in the plastic containers to absorb distilled water for 8 h then all of them were placed into a plastic sheet chamber located within the experimental glasshouse where a relative humidity in the chamber was maintained at approximately 90-100% with a natural day light intensity of approximately 70%. All petioles of the detached leaves were individually planted into sand particles in each plastic pouch and they were kept in a plastic sheet chamber. Nutrient solution was adequately added where appropriate. An inoculation of spores of the disease was carried out at day 29 after emergence.

With the preparation on growth of cowpea seedlings with the use of plastic growth pouches technique, the method of Somasegaran and Hoben (1994) was used where a modified Long Ashton solution was carefully prepared with an additional amount of nitrogen fertiliser of 240 ppm (Summerfield *et al*., 1977). Transparent plastic bags (heat resistance) with a dimension of a 10×16 cm in width and length were used. Each bag was divided into 3 equal sizes by electrical plastic sealing machine, i.e., 3 small separated plastic bags were attained. Each small bag contained a piece of brown tissue paper in it with a dimension of approximately 3×10 cm in width and length being inserted into each plastic bag for use in absorbing the added amount (5.00 mL) of nutrient solution into the bags for use in germinating cowpea seeds. In each bag, a small opened point fitted seed of cowpea was carried out at top of each bag. After the added amount of nutrient solution was carried out, sterilized seeds of the cowpea cultivars (sterilization was carried out in a 10% Chlorox solution, i.e., a 10% NaOCl concentration in distilled water for 10 min) were inserted into each bag leaving about one half of its length of individual seed at top of the bag then each seed was ready for germination process and finally placed all of the germinating bags into a plastic chamber where a 100% relative humidity was maintained. Thus germination process of seeds was started then 5 mL of nutrient solution was added to each bag when needed (being observed by naked eye) to assure adequate moisture content in the germinating bag. At 4 days after emergence, initial primary leaves were removed and then pulled out the seedlings together with brown paper away and placed them into plastic pouches where each plastic pouch was added with nutrient solution and allowed the seedlings to grow until day 28 after emergence under glasshouse conditions then transferred the seedlings into plastic growth pouches then placed them under a plastic chamber of controlled relative humidity (100%). An inoculation of spores of the disease was carried out according to their respective treatments at a followed day after the transfer of the seedlings into the plastic chamber. An inoculation of the spores of the disease was carried out at the same time as that of the detachd leaf technique, i.e., at day 29 after emergence.

The spores of pathogenic disease of *Pseudocercospora cruenta* (Sacc.) Deighton were collected from the severely infected leaves of KKU25 cowpea plants, which were allowed to grow under glasshouse conditions approximately two months prior to the commencing of the experiment. The collected infected leaves were submersed in distilled water and then roughly stirred in order to pull out spores of the disease from leaves. The collected spores were counted to attain their respective concentrations for use in the experiment. The counting was carried out with the use of Haemacytometer. The rearing of the plant samples in the plastic chamber under the glasshouse conditions was carried out with a special care, i.e., the percentage of relative humidity was kept at 90-100%, night and day temperatures of 28±2 to 33±4°C, respectively. For most of the detached leaves, 7 days after planted, some roots had developed and the leaves were able to absorb nutrients for their survival and then plastic sheets covered the chamber was removed so that the plant samples were able to expose themselves freely to the environment under the glasshouse conditions. The water table being used as a sub-irrigation system in the plastic trays for detached leaf technique was kept at 1 cm, whilst additional amount of nutrient solution in plastic bags of cowpea seedlings was also adequately kept at its base of roots. A high frequency of inspection was carried out daily to assure adequate supply of both water and nutrients. At the same time the control of environmental conditions was also carefully inspected.

Recordings on the amount of infected disease were carried out at 7 day intervals starting from day 7 until day 28 after an inoculation of the pathogenic disease to the plant samples, i.e., four times of the recording periods were used. The disease incidences (%) were recorded based on the assumption that the most susceptible cultivar (KKU25) has its disease incidences of 100% whilst severity recorded scores were accounted from 1-5, i.e., 1 = none infection; 2 = 1-3 spots on leaf surface with a diameter lesser than 5 mm; 3 = spot numbers greater

than 3, each with a diameter lesser than 5 mm; 4 = spots of dark grayish spores with a diameter more than 5 mm where at least one spot appears on primary leaf surface and three spots per leaf of tri-foliage leaves and 5 = more than three spots of dark grayish spores on leaf surface where it appears on primary leaf numbers 1-4 (Sinsiri *et al*., 2006). The recorded results were statistically analysed using a two ways analysis of variance and Duncan's Mulitiple Range Test (DMRT) of an MSTAT-C Computer Programme (Nissen, 1989).

RESULTS

Disease incidences and severity of infection: For the effect due to cultivars (factor A), the results showed that percentages of disease incidences were initially found during the first sampling period after the inoculation of the disease where KKU25 gave percentages of 25.27, 27.68, 29.00 and 29.95 for days 7, 14, 21 and 28, respectively whereas KVC7 and IT81D-1228-14-1 cultivars were not infected by pathogenic disease throughout the experimental period (Table 1). The differences were large and highly significant. With degrees of severity, a similar trend to that of the infected percentages was found, i.e., KKU25 gave severity scores of 2.47, 2.53, 4.09 and 4.78 for days 7, 14, 21 and 28, respectively, whilst IT81D-1228-14-1 cultivar gave some small values of the infected disease only at days 21 and 28 with values of 1.38 and 1.68, respectively. Both IT81D-1228-14-1 and KVC7 cultivars were similar. However, the differences found among the three cultivars of the four sampling periods were large and highly significant.

With the effect due to both testing techniques, i.e., detached leaf and plastic growth pouches (factor B), the results showed that percentages of disease incidences were found at the initial sampling until the final sampling periods where detached leaf technique gave percentages of 12.64, 12.81, 15.73 and 16.29 for days 7, 14, 21 and 28, respectively. Whilst plastic growth pouches technique gave percentages of 4.21, 5.65, 3.91 and 4.29 for days 7, 14, 21 and 28, respectively. The differences between detached leaf technique and plastic growth pouches were large and highly significant where detached leaf technique gave a severe percentage of infection higher than plastic growth pouches (Table 2). For severity levels, the results showed that detached leaf technique gave values of 1.5, 1.52, 2.23 and 2.54 for days 7, 14, 21 and 28, respectively. Whilst plastic pouches technique gave severity values of 1.48, 1.5, 2.08 and 2.44 for days 7, 14, 21 and 28, respectively. The differences between detached leaf and plastic growth pouches techniques on severity of the disease were not statistically significant.

With the effect on the infectionof the disease due to four levels of pathogenic spores (factor C), the results showed that at day 7 after inoculation, an increase in level of inoculated spores highly increased percentages of disease incidences over level 1 (0 colony forming unit) with values ranged from 0.00 to 18.12% for level 1 to level 4, respectively (Table 3). At day 14 up to day 28 after inoculation, an increase in level of inoculated spores highly increased percentages of disease incidences yet the differences between level 2 and level 3 of the sampling periods of days 14 and 21 were relatively small and not

Table 1: Percentages and severity levels of disease infection with time (days after inoculation) due to cultivars (factor A) of KVC7, IT81D-1228-14-1 and KKU25 cowpea cultivars

	Disease incidence (%)				Severity levels (1-5)			
	7	14	21	28	7	14	21	28
Cultivars	(DAI)				(DAI)			
KVC7	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	1.0^{b}	1.0^{b}	1.0^{b}	1.0^{b}
IT81D-1228-14-1	0.00^{b}	0.00^{b}	0.46^{b}	0.91^{b}	1.0^{b}	1.0^{b}	1.38^{b}	1.68^{b}
KKU25	25.27^{a}	27.68^{a}	29.00^{a}	29.95^{a}	2.47^{a}	2.53^{a}	4.09^{a}	4.78^{a}
F-test	**	**	**	**	**	**	**	**
CV (%)	15.83	22.39	14.62	25.43	6.85	6.76	12.36	8.53

Letter (s) within columns indicate significant differences of DMRT at probability **: p = 0.01, DAI: Days after inoculation of the disease

Table 2: Percentages and severity levels of disease infection with time (days after inoculation) due to detached leaf and plastic growth pouches techniques (factor B) of KVC7, IT81D-1228-14-1 and KKU25 cowpea cultivars

	Incidence (%)				Severity levels (1-5)			
	7	14	21	28	7	14	21	28
Screening techniques	(DAI)				(DAI)			
Detached leaf	12.64^{a}	12.81^{a}	15.73^{a}	16.29^{a}	1.5	1.52	2.23	2.54
Plastic pouches	4.21^{b}	5.65^{b}	3.91^{b}	4.29^{b}	1.48	1.5	2.08	2.44
F-test	**	**	**	**	NS	NS	NS	NS
CV (%)	15.83	22.39	14.62	25.43	6.85	6.76	12.36	8.53

Letter (s) with in columns indicate significant differences of DMRT at probability **: p = 0.01, NS Non Significant, DAI: Days after inoculation of the disease

Table 3: Percentages and severity levels of disease infection with time (days after inoculation) due to disease concentrations (factor C) of KVC7, IT81D-1228-14-1 and KKU25 cowpea cultivars

	Incidence (%)				Severity levels (1-5)			
	7	14	21	28	7	14	21	28
Disease concentrations	(DAI)				(DAI)			
0 cfu mL^{-1}	0.00^{d}	0.15^{c}	0.44^{c}	1.40^{c}	1.00^{b}	1.04^{b}	1.33^{c}	2.04^{b}
1×10^{4} cfu mL^{-1}	5.89^{c}	7.34^{b}	9.08^{b}	9.63^{b}	1.63^{a}	1.67^{a}	2.21^{b}	2.63^{a}
5×10^{4} cfu mL^{-1}	9.69^{b}	10.64^{b}	11.37^{b}	11.58^{b}	1.67^{a}	1.67^{a}	2.46^{a}	2.63^{a}
1×10^{5} cfu mL^{-1}	18.12^{a}	18.78^{a}	18.37^{a}	18.56^{a}	1.67^{a}	1.67^{a}	2.63^{a}	2.67^{a}
F-test	**	**	**	**	**	**	**	**
CV (%)	15.83	22.39	14.62	25.43	6.85	6.76	12.36	8.53

Letter(s) within columns indicate significant differences of DMRT at probability **: p = 0.01, DAI: Days after inoculation of the disease; cfu mL^{-1} : Concentration of disease forming units mL^{-1} of distilled water

Table 4: Percentages and severity levels of disease infection with time (days after inoculation) due to an interaction of the three factors, i.e., A×B×C of KVC7, IT81D-1228-14-1 and KKU25 cowpea cultivars

	Incidence (%)				Severity levels (1-5)			
	7	14	21	28	7	14	21	28
A×B×C interactions	(DAI)				(DAI)			
KVC7×DLT×(0)	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
KVC7×DLT×(1×10^{4})	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
KVC7×DLT×(5×10^{4})	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
KVC7×DLT×(1×10^{5})	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
KVC7×PGP×(0)	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
KVC7×PGP× (1× 10^{4})	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
KVC7×PGP×(5×10^{4})	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
KVC7×PGP×(1×10^{5})	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
IT81D-1228-14-1×DLT×(0)	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
IT81D-1228-14-1×DLT×(1×0^{4})	0.00^{e}	0.00^{d}	0.20^{fg}	1.01^{fg}	1.00	1.00	1.00	2.00^{c}
IT81D-1228-14-1×DLT×(5×0^{4})	0.00^{e}	0.00^{d}	0.82^{fg}	1.22^{fg}	1.00	1.00	1.75	1.75^{c}
IT81D-1228-14-1×DLT×(1×10^{5})	0.00^{e}	0.00^{d}	0.81^{fg}	1.62^{fg}	1.00	1.00	1.75	2.00^{c}
IT81D-1228-14-1×PPT×(0)	0.00^{e}	0.00^{d}	0.00^{g}	0.00^{g}	1.00	1.00	1.00	1.00^{d}
IT81D-1228-14-1×PPT×(1×10^{4})	0.00^{e}	0.00^{d}	0.00^{g}	0.60^{g}	1.00	1.00	1.00	1.75^{c}
IT81D-1228-14-1×PPT×(5×10^{4})	0.00^{e}	0.00^{d}	0.62^{fg}	1.44^{fg}	1.00	1.00	1.50	2.00^{c}
IT81D-1228-14-1×PPT×(1× 10^{5})	0.00^{e}	0.00^{d}	1.23^{fg}	1.41^{fg}	1.00	1.00	2.00	2.00^{c}
KKU25×DLT×(0)	0.00^{e}	0.90^{d}	2.04^{f}	6.16^{e}	1.00	1.25	2.25	4.75^{a}
KKU25×DLT×(1×10^{4})	24.45^{c}	23.86^{c}	41.59^{c}	42.20^{c}	3.00	3.00	5.00	5.00^{a}
KKU25×DLT×(5×10^{4})	43.48^{b}	42.77^{b}	53.07^{b}	53.08^{b}	3.00	3.00	5.00	5.00^{a}
KKU25×DLT×(1×10^{5})	83.69^{a}	86.15^{a}	90.16^{a}	90.16^{a}	3.00	3.00	5.00	5.00^{a}
KKU25×PPT×(0)	0.00^{e}	0.00^{d}	0.61^{fg}	2.26^{fg}	1.00	1.00	1.75	3.50^{b}
KKU25×PPT×(1×10^{4})	10.86^{e}	20.18^{c}	22.71^{e}	23.96^{e}	2.75	3.00	4.25	5.00^{a}
KKU25×PPT×(5×10^{4})	14.67^{d}	21.08^{c}	23.73^{e}	23.73^{e}	3.00	3.00	4.50	5.00^{a}
KKU25×PPT×(1×10^{5})	25.00^{c}	26.51^{c}	28.03^{d}	28.03^{d}	3.00	3.00	5.00	5.00^{a}
F-test	**	**	**	**	NS	NS	NS	**
CV (%)	15.83	22.39	14.62	25.43	6.85	6.76	12.32	8.53

Letter (s) within columns indicate significant differences of DMRT at probability **: p = 0.01, NS: Non significant; DAI: Days after inoculation of the disease; DLT: Detached Leaf Technique, PPT: Plastic growth pouches technique

statistically significant and thereafter with the highest level (level 4) of inoculated spores, the increases were large and highly significant in all sampling periods with values of 18.78, 18.37 and 18.56 for days 14, 21 and 28, respectively. For severity levels, the results showed that an increase in level of inoculated spores highly increased level of severity up to inoculated level 2 with values of severity level of 1.63, 1.67, 1.33 and 2.04 for days 7, 14, 21 and 28, respectively and thereafter a small increase was found only with the sampling period of day 21 whilst the rest, in most cases, remained the same. The highest inoculated level of spores of the disease (level 4) gave severity levels of 1.67, 1.67, 2.63 and 2.67 for the sampling periods of days 7, 14, 21 and 28, respectively.

Interactions among the three factors: To justify the interactions among the three factors (a, b, and c) with a clearer image, a further analysis was carried out. The results on the interactions among the three factors, i.e., cultivars (a), techniques of detached leaf and plastic growth pouches techniques (b) and the various concentrations of the spores of the disease being inoculated (c) revealed that infected disease incidences (%) were severely found with KKU25 cultivar at the

highest concentration of inoculated spores (1×10^5 forming units mL^{-1}) with a value of 83.69 % (detached leaf technique) whereas IT81D-1228-14-1 cultivar gave 0.00 % (Table 4). However, with the results on severity levels, it turns out that a highly significant was found at day 28 where severity level of KKU25 cultivar, in most cases, reached a value of 5 whilst other sampling periods were not statistically significant.

DISCUSSION

This investigation aimed to compare the effectiveness of the results derived from the application of both screening techniques, i.e., detached leaf and plastic growth pouches with respect to cowpea cultivars and concentrations of spores of the disease being inoculated in the experiment where the results on disease incidences (%) and severity levels (1-5) were used. With respect to the effect due to cultivars (factor A), the Table 4 showed that KVC7 and IT81D-1228-14-1 cowpea cultivars were not affected by different inoculated concentrations of spores, i.e., no sign of infection of the disease was found. However, with KKU25 cultivar, the infection was relatively large and highly significant. The results confirm the results of thee earlier experiment of Sinsiri *et al.* (2006) where they reported that KVC7 and IT81D-1228-14-1 cultivars possessed immune and high resistant properties against the disease, respectively, whereas KKU25 cultivar ranked as a susceptible cultivar against the disease. A similar result was found with severity levels where KKU25 was severely infected by the disease and the differences were highly significant. When it comes to the effect due to detached leaf and plastic pouches techniques (factor B), the results showed that percentages of disease incidences of detached leaf technique were much higher than plastic growth pouches technique and the differences were highly significant. This could possibly be attributable to leaf sizes of individual leaves (leaf area), i.e., the leaves of the cowpea seedlings, in all cases, were relatively smaller for plastic growth pouches technique than detached leaf technique. The smaller leaf areas of seedlings grown with the use of plastic growth pouches could have been due to a smaller amount of nutrient taken up by the seedlings. It could have been possible that the use of soil as a medium could facilitate the growth of cowpea plants better than growing with nutrient solution. It was found that the use of soil as a growth medium for detached leaf technique gave much higher amount of infection than plastic growth pouches. Therefore, detached leaf technique could possibly be the method suited most for screening of diseases in plants rather than plastic growth pouches technique. However, it has been reported that plastic growth pouches technique was suited most for use in a study on growth and development of plant roots (Summerfield *et al.*, 1977; Somasegaran and Hoben, 1994; Greenville *et al.*, 1986; Chambers *et al.*, 1998).

With the effect due to disease concentrations being applied (factor C), the results showed that an increase in disease concentration being inoculated increased percentages of disease incidences although both concentrations (1×10^4 and 5×10^4 forming units mL^{-1}), in most cases, were not statistically different from each other. It was found that leaves of the cowpea plants inoculated at a concentration of 1×10^5 forming units mL^{-1} gave a plentiful amount of infection, which may be considered too high thus concentrations of 1×10^4 and 5×10^4 forming units mL^{-1} may be of appropriate concentrations. For severity levels, it turned out that in most sampling days, severity levels were similar but highly significant when compared with control (0 forming units mL^{-1}). Therefore, a concentration rate of 1×10^4 forming units mL^{-1} may be considered applicable for further experiments. With the results on the interactions among the three factors (A×B×C), it was found that a severe result was found with KKU25 cultivar, particularly on day 28 after inoculation, whilst other cultivars gave a minute amount of disease incidences where detached leaf technique gave much higher percentages of disease incidences compared with plastic growth pouches technique, thus detached leaf technique should be of a greater advantage than plastic growth pouches technique although both techniques may be equally used but perhaps depending on scientists preferences.

CONCLUSION

Among the cowpea cultivars being used for this study, i.e., KVC7, IT81D-1228-14-1 and KKU25, KKU25 was severely infected by the inoculated pathogenic disease of *Pseudocercospora cruenta* (Sacc.) Deighton where the degree of infection was much higher than the rest and the differences were highly significant. Detached leaf technique gave much better results than plastic growth pouches technique, thus detached leaf technique should be chosen for further experiments rather than plastic growth pouches technique. The most applicable rate of disease concentration for use in inoculating the pathogenic disease to the plant samples should be at a concentration of 1×10^4 forming units mL^{-1} of distilled water. An Interaction found among the three factors (A×B×C) with respect to the three cultivars was severely found at day 28 after inoculation but only with KKU25 cultivar alone.

ACKNOWLEDGMENTS

The authors wish to thank the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University for financial support and seeds of the three cowpea cultivars. Thanks are also due to faculty members and personnel of the Department of Agricultural Technology for their kind assistance during this study was carried out.

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