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An Evaluation on Intensity of Infection of Pseudocercospora Leaf Spot Disease of Cowpea Cultivars (*Vigna unguiculata* L. Walp), With Respect to Infector Rows, Dates of Inoculation and Cultivars, Grown Under Field Conditions in Northeast Thailand

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Abstract: This investigation was carried out at the Experimental Farm, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand to evaluate intensity of infection of Pseudocercospora Leaf Spot disease of cowpea cultivars, grown under field conditions with the use of Yasothon soil series (Oxic Paleustults). The experimental design used was a strip split plot design with four replications. The experiment consisted of three factors, i.e., with and without infector rows (Factor A), with and without inoculation and inoculated dates, i.e. none inoculation, inoculated at days 15 and 30 after sowing of seeds in main plots (Factor B) and three cowpea cultivars of KVC7, KKU25 and IT81D-1228-14-1 (Factor C). The results showed that the use of infector rows of cowpea plants (KKU25) being sown at 15 days before sowing seeds of cowpea cultivars in main plots gave a similar intensity of disease as those infector rows being sown at 30 days before sowing seeds of cowpea cultivars in main plots but significantly higher than without infector rows. Pathogenic disease being inoculated to the cowpea plants at 15 days after the sowing of seeds in main plots gave significantly higher intensity of disease than none inoculation. The establishment of infector rows at 15 days ahead of the sowing of seeds in main plots together with an inoculation of the disease at 15 days after the sowing of seeds in main plots gave the best results in evaluating amount of the infected disease. Thus infector rows surrounded the main plots are required whenever trials on disease evaluation are taken place.

Key words: Cowpea cultivars, Pseudocercospora leaf spot disease, disease intensity, seed yields

INTRODUCTION

It is generally recognized by a number of plant breeders that selection for high performance of economic crop cultivars against diseases should be carried out by introducing diseases to the crops where the results of the infection of the diseases could be evidently assessed, thus outstanding crop cultivars could be chosen for further breeding programmes. The methods to be applied for selection should be of low inputs, easy to evaluate and be able to provide its outcome similar to the results derived from the plants being grown under natural field conditions. Some plant breeders have obviously chosen to sow seeds of poor resistant cultivars (being used as infector rows) ahead of others aiming to obtain some certain amount of infected disease where the infected plants could be used as a source of pathogenic disease then the sowing of seeds of other cultivars to be selected could be carried out within a certain period of time. In doing this way, plant breeders could possibly foresee the outbreak of the disease with other introduced cultivars within a few weeks if not earlier. Nakawuka and Adipala (1997) had sown a high susceptible cowpea cultivar to Sphaceloma scab disease as infector rows 10 days ahead of other cultivars to be tested. They reported that at 15 days after the sowing of the tested cultivars, some certain amounts of infected cowpea plants were able to spread out disease along the rows of the various tested cultivars, i.e., the plants within the infector rows were able to introduce more pathogenic spores to the tested plants. They stated that the application of infector rows could largely assist the trial where they were able to assess successfully the infection of the disease among the 25-cowpea cultivars. Similarly, Adejumo *et al.* (2001) applied infector rows technique to assess Leaf Smut disease infection with the use of 37-cowpea cultivars where IT86D-1010 cultivar was used as 100% infection with a highest severity level of 5 (severity levels ranged from 1-5). They reported that the 37-cowpea cultivars were successfully identified into 3 groups, i.e., no infection,

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resistant and susceptible cultivars. Thus the use of infector rows technique in assessing disease infection has proved to possess reliable results for plant breeders to attain outstanding gene of the cultivars against the disease, which could be useful for further breeding programme.

Another interesting point lies on plant ages when inoculation has to be carried out by mechanical means or even being introduced by airborne diseases. The plant ages may affect intensity of infection, thus Fery and Dukes (2002) carried out experiment with the use of 2-cowpea cultivars on the infection of Southern Blight (*Sclerotium rolfsii* Sacc.) where they had 4 levels of inoculation, i.e., control, inoculated at days 35, 45 and 55 after sowing with the use of spores of 20 sclerotia for each plant. They found that cowpea plants when inoculated at early ages, the disease could damage the growth and yield of the crop plants more severely than being inoculated during the later periods, i.e., at 45 and 55 days after sowing. Similarly, McCallum and Tekauz (2002) with 4-barley cultivars on the infection of Head Blight disease of the pathogenic organisms of *Fusarium graminearum* Schwabe. They did inoculate spores of the disease into three stages of growth, i.e., right after the appearance of flowers above flag leaf and then at days 7 and 14 after the initial inoculation. They reported that intensity of severity of infection of the initial inoculation was significantly higher than the subsequent inoculations, i.e., intensity of severity significantly declined with the advanced in age of the cowpea plants. Another experiment carried out by Reid *et al.* (2002) with the use of maize (*Zea mays* L.) as indicator plants on Ear Rot disease. They reported that inoculated of disease spores at 4-7 days after the appearance of silks gave the highest intensity of infection and other later inoculations gave a significant decline in severity level of the disease. Therefore, it is of important value to carry out field experiment with the use of cowpea cultivars in order to justify effects of infector rows and inoculated ages of the plants in relation to intensity of infection of the disease so that the attained information may be useful for plant breeders and growers of the cowpea plants.

MATERIALS AND METHODS

This field experiment was carried out in the rainy season of the 2004 (July-October) at the Experimental Farm, Khon Kaen University, Khon Kaen, Northeast Thailand to investigate effects of infector rows (Factor A), inoculation dates (Factor B) and cowpea cultivars (Factor C) on the intensity of infection of Pseudocercospora Leaf Spot disease of cowpea cultivars. Yasothon soil series (Oxic Paleustults) was used. For Factor A, it consisted of three different periods of time in sowing seeds of infector rows (KKU25 cowpea cultivar was used for infector rows apart from being used as an actual member of the tested cultivars, i.e., KVC7 and IT81D-1228-14-1) that is: no infector rows surrounded the experimental plots, sown seeds of KKU25 cowpea cultivar as infector rows surrounded the plots at 15 and 30 days ahead of time before the sowing of seeds of KVC7, IT81D-1228-14-1 and also KKU25 (the tested cultivars) in the experimental plots. Factor B, it included no inoculation, inoculated at 15 and 30 days after the sowing of cowpea seeds of the 3-cultivar of Factor C. Factor C, it composed of KVC 7, IT81D-1228-14-1 and KKU25 cowpea cultivars. The experimental design used was a Strip Split Plot Design and each treatment was duplicated four times. The recommended rate of spores (5×10^4 colony forming units mL^{-1} of distilled water) of the pathogenic disease of *Pseudocercospora cruenta* (Sacc.) Deighton was used (Sinsiri and Laohasiriwong, 2008). The plot size used was a 2×4 m with a path of 1.5 m in between the plots. The sowing distance used was a 50×25 cm between rows and within rows, respectively. For each drill, a few seeds of their respective cowpea cultivars were sown directly into soil by hand and 1 week after immergence seedlings were thinned out leaving only one seedling $drill^{-1}$. This was also carried out with those of infector rows surrounded the plots. Weeding was carried out by hand twice, i.e. the first one was done at 20 days after sowing where a complete chemical fertilizer 15-15-15 (NPK) at a rate of 156.25 kg ha^{-1} was evenly applied by hand and the second one was carried out at 35 days after sowing.

An inoculation of spores of the disease to cowpea seedlings was carried out at 15 and 30 days after the sowing of seeds in the replicated plots, i.e., approximately 12 and 27 days after emergence. The disease infection determination was carried out at days 37, 44, 51, 58 and 65 after sowing. The method in determining disease incidences and severities in cowpea leaves was carried out with the use of the method described by Adejumo *et al.* (2001) and Sinsiri *et al.* (2006) where the infected incidence of the disease found with KKU25 cultivar (the weakest cultivar) was given a score of 100% and a severity range of 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 greater than 5 mm where at least one spot appears on primary leaf surface and three spots $leaf^{-1}$ 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. The results of day 65 after sowing on

severity level were not included due to a severe damage to the plants. The recorded results were statistically analysed using two ways analysis of variance and Duncan's Multiple Range Test (DMRT) of an MSTAT C Computer Programme (Nissen, 1989).

RESULTS

Disease incidence and severity due to infector rows With Factor A (infector rows), the results showed that disease incidences (%) were similar in each sampling period starting from day 37 up to day 58 before sowing of seeds in main plots. However, at day 65 disease incidences were large and statistically significant where both infector rows of 15 and 30 days before sowing gave significantly higher percentages than none infector row but both gave a similar significant value of 24.90 and 25.67% for infector rows of 15 and 30 days before sowing, respectively (Table 1). For severity level (1-5), the results revealed that at day 37 before sowing, severity level ranged from 1.75 up to 2.31 for none infector row and infector row being sown at 30 days before sowing, respectively. The differences were large and highly significant yet infector row being sown at 15 days before sowing gave a similar level as none infector row. At 44 days before sowing of seeds in main plots, the results showed that severity values ranged from 1.89 to 2.22 for none infector row and infector row of 30 days before sowing of seeds in main plots, respectively. The differences were large and highly significant but none infector row was similar to infector row of 15 days before sowing of seeds in main plots, whilst with the subsequent sampling periods of 52 and 58 days before sowing of seeds in main plots were similar and not statistical significant. Seed yields were similar in all treated infector rows with values ranged from 652 to 783 kg ha^{-1} for infector row of 30 days before sowing of seeds in main plots, respectively.

Effect due to inoculated dates: For Factor B (inoculation dates), the results showed that at day 37 disease incidences of the none inoculation were much smaller than inoculated at days 15 and 30 after sowing of seeds in main plots with values ranged from 1.78 to 17.92% for none inoculation and inoculated at 15 days after sowing of seeds in main plots, respectively (Table 2). The differences were large and highly significant. A similar significant trend on disease incidences was found with most sampling periods from day 44 up to day 65 after sowing of seeds in main plots, i.e., in most cases, those inoculated at days 15 and 30 after sowing of seeds in main plots, were highly significant where both inoculated dates were similar with values ranged from 11.64 to 26.47% for none inoculation and inoculated at 15 days after sowing of seeds in main plots. With severity level, the results showed that at days 37 and 44 after sowing of seeds in main plots, a severe result was found with the one inoculated at day 15 after sowing of seeds in main plots with values ranged from 1.75 to 2.33 for none inoculation and inoculated at day 15 after sowing of seeds in main plots where inoculated at day 30 gave a similar level as none inoculation. At days 51 and 58 after sowing of seeds in main plots, severity levels were similar in all none inoculated and both inoculated at days 15 and 30 after

Table 1: Disease incidences and severity levels with time as affected by treatment without infector rows and with infector rows of seeds sown at 15 and 30 days before sowing of cowpea seeds in main plots (Factor A) and final seed yields of cowpea cultivars

Factor A with and without infector rows	Disease incidence (%)					Severity levels (1-5)				Seed yields (kg ha^{-1})
	37 DAS	44 DAS	51 DAS	58 DAS	65 DAS	37 DAS	44 DAS	51 DAS	58 DAS	
Without infector rows	11.29	12.33	15.69	14.81	12.96b	1.75b	1.89b	2.28	2.64	783.00
With infector rows at 15 DBS	12.79	13.93	16.54	15.15	24.90a	1.92b	2.00ab	2.19	2.67	782.00
With infector rows at 30 DBS	7.95	12.36	14.28	17.63	25.67a	2.31a	2.22a	2.31	2.67	652.00
F-test	NS	NS	NS	NS	*	**	**	NS	NS	NS
CV (%)	35.71	36.31	31.14	24.61	32.57	11.51	17.67	14.34	3.62	26.02

Letter(s) within column indicate Least Significant Differences (LSD) of Duncan's Multiple Range Test (DMRT) of probability (p)**0.01, *0.05, DAS: Days After Sowing of seeds in main plots, DBS: Days Before Sowing of seeds in main plots, NS: None Significant, CV: Percentage of covariance

Table 2: Disease incidences and severity levels with time as affected by time of inoculation of the disease (Factor B), i.e., no inoculation, inoculated at 15 and 30 days before sowing of cowpea seeds in main plots and final seed yields of cowpea cultivars

Factor B inoculation dates	Disease incidence (%)					Severity levels (1-5)				Seed yields (kg ha^{-1})
	37 DAS	44 DAS	51 DAS	58 DAS	65 DAS	37 DAS	44 DAS	51 DAS	58 DAS	
None inoculation	1.78b	2.78c	4.31b	5.88b	11.64b	1.75b	1.97ab	2.22	2.64	804.00
Inoculation at 30 DBS	12.35a	24.90a	24.57a	25.06a	25.42a	1.89b	1.83b	2.22	2.67	714.00
Inoculation at 15 DBS	17.92a	10.94b	17.64a	16.65a	26.47a	2.33a	2.31a	2.33	2.67	699.00
F-test	**	**	**	**	**	**	**	NS	NS	NS
CV (%)	35.71	36.31	31.14	24.61	32.57	11.51	17.67	14.34	3.62	26.02

Letter(s) within column indicate Least Significant Differences (LSD) of Duncan's Multiple Range Test (DMRT) at probability (p) **0.01, NS: None Significant, DAS: Days After Sowing of seeds in main plots, DBS: Days Before Sowing of seeds in main plots, CV: Percentage of covariance.

sowing of seeds in main plots. Seed yields were highest with non inoculation followed by inoculated at days 30 and 15 after sowing of seeds in main plots with values of 804, 714 and 699 kg ha^{-1}, respectively. There was no significant effect on seed yields found due to none inoculation and both inoculated plants (days 15 and 30 after sowing of seeds in main plots).

Effect due to cultivars: With the effect due to cultivars (Factor C), the results showed that disease incidences (%) were highest with KKU25, whilst KVC7 and IT81D-1228-14-1 cultivars were similar in all sampling periods. The differences among the cultivars in all sampling periods were large and highly significant where the percentages found at day 65 after sowing seeds in main plots ranged from 0.00 to 61.69 for KVC7 and KKU25, respectively (Table 3). For severity levels, the results revealed that a similar trend to disease incidences was found, i.e. the highest infected level was found with KKU25, whilst KVC7 and IT81D-1228-14-1 were similar. The differences due to severity levels were large and highly significant where at day 58 after sowing of seeds in main plots gave values ranged from 1.00 to 5.00 for KVC7 and KKU25, respectively.

Interactions between inoculate date and cultivar: For the results on an interaction between inoculated dates and cultivars (B×C), the results showed that with both non inoculated and with inoculated, disease incidences of susceptible cultivar (KKU25) were severely infected by the disease compared with other two cultivars (KVC7 and IT81D-1228-14-1), particularly with the use of infector rows (Table 4). At 37 days after sowing of seeds in main plots, the results revealed that none inoculation gave no infection with the resistant cultivars (KVC7 and IT81D-1228-14-1) whilst KKU25 attained 5.33% of the disease incidence. A severe disease incidence was found with inoculation at 30 days after sowing where KKU25 gave disease incidences up to 37.04% whereas other two cultivars did not infect by the disease. A greater intensity of disease infection greater than inoculated at 30 days after sowing was found with the inoculation at 15 days after sowing where KKU25 gave a value of infection up to 53.75%. A similar trend on disease incidence was found in all subsequent sampling periods at days 44 up to day 65 after sowing. The differences in each sampling period were large and highly significant. With severity levels, the results showed that at day 37 after sowing, severity level was relatively small with none inoculation compared with both dates of inoculation (15 and 30 days after sowing) where inoculated date of 15 days after sowing showed highest value of severity level than inoculated at 30 days after sowing with values of 5.00 and 3.67, respectively. Other sampling periods of days 44, 51 and 58 after sowing gave a similar trend as that of disease incidences, i.e., KKU25 cultivar was severely infected by the disease but day 30 was relatively lesser than day 15 after sowing. The differences were large and highly significant.

Table 3: Disease incidences and severity levels with time as affected by different cowpea cultivars (Factor C) and final seed yields of cowpea cultivars

	Disease incidence (%)					Severity levels (1-5)				
Factor C cultivars	37 DAS	44 DAS	51 DAS	58 DAS	65 DAS	37 DAS	44 DAS	51 DAS	58 DAS	Seed yields (kg ha^{-1})
KVC7	0.00b	0.00b	0.00b	0.00b	0.00b	1.00b	1.00b	1.00b	1.00b	728.00b
IT81D-1228-14-1	0.00b	0.00b	0.00b	1.22b	1.83b	1.00b	1.00b	1.00b	2.00b	982.00a
KKU25	32.04a	38.63a	46.51a	46.36a	61.69a	3.97a	4.11a	4.78a	5.00a	597.00c
F-test	**	**	**	**	**	**	**	**	**	**
CV (%)	35.71	36.31	31.14	24.61	32.57	11.51	17.67	14.34	3.62	26.02

Letter(s) within column indicate Least Significant Differences (LSD) of Duncan's Multiple Range Test at probability (p) **0.01, DAS: Days After Sowing of seeds in main plots, DBS: Days Before Sowing of seeds in main plots, CV: Percentage of covariance

Table 4: An interaction between B×C (inoculation time×cultivars) on disease incidences and severity levels with time

	Disease incidence (%)					Severity levels (1-5)				
B×C interactions	37 DAS	44 DAS	51 DAS	58 DAS	65 DAS	37 DAS	44 DAS	51 DAS	58 DAS	Seed yields (kg ha^{-1})
None inoc×KVC7	0.00d	0.00d	0.00d	0.00d	0.00d	1.00d	1.00d	1.00	1.00	806.00bc
None inoc×IT81D-1228-14-1	0.00d	0.00d	0.00d	1.33d	1.58c	1.00d	1.00d	1.00	2.00	875.00b
None inoc×KKU25	5.33c	8.33c	12.92c	16.29c	33.33b	3.25c	3.92b	4.67	4.92	731.00cd
Inoc. 30 DBS×KVC7	0.00d	0.00d	0.00d	0.00d	0.00d	1.00d	1.00d	1.00	1.00	655.00d
Inoc. 30 DBS×IT81D-1228-14-1	0.00d	0.00d	0.00d	1.25d	1.88c	1.00d	1.00d	1.00	2.00	938.00a
Inoc. 30 DBS×KKU25	37.04b	74.71a	73.71a	73.92a	74.38a	3.67b	3.50c	4.67	5.00	515.00e
Inoc. 15 DBS×KVC7	0.00d	0.00d	0.00d	0.00d	0.00d	1.00d	1.00d	1.00	1.00	723.00cd
Inoc. 15 DBS×IT81D-1228-14-1	0.00d	0.00d	0.00d	0.00d	2.04c	1.00d	1.00d	1.00	2.00	827.00b
Inoc. 15 DBS×KKU25	53.75a	32.83b	52.92b	48.88b	77.38a	5.00a	4.92a	5.00	5.00	545.00e
F-test	**	**	**	**	**	**	**	NS	NS	**
CV (%)	35.71	36.31	31.14	24.61	32.57	11.51	17.67	14.34	3.62	26.02

Letter(s) within column indicate Least Significant Differences (LSD) of Duncan's Multiple Range Test (DMRT) at probability of (p) **0.01, Inoc.: Inoculation, DAS days after sowing of seeds in main plots, DBS: Days Before Sowing of seeds in main plots, DAS: Days After Sowing of seeds in main plots, Inc: Inoculation, NS: None Significant, CV: Percentage of covariance

Table 5: Interactions among factors (A×B×C), i.e., with and without inoculation (A)×inoculation dates (B)×cowpea cultivars (C) on disease incidence (%), severity levels (1-5) and seed yields (kg ha^{-1}) of cowpea cultivars as influenced by infector rows and without infector rows

	Disease incidence (%)					Severity levels (1-5)				Seed yields
A×B×C interactions	37 DAS	44 DAS	51 DAS	58 DAS	65 DAS	37 DAS	44 DAS	51 DAS	58 DAS	(kg ha^{-1})
None inf×none ino×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	870
None inf×none ino×IT81D-1228-14-1	0.00a	0.00	0.00	1.50	1.38	1.00d	1.00d	1.00	2.00	993
None inf×none ino×KKU25	2.25a	3.25	9.38	13.00	17.88	1.75c	3.00c	5.00	4.75	868
None inf×inoc 30 DAS×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	635
None inf×inoc 30 DAS×IT81D-1228-14-1	0.00a	0.00	0.00	1.25	1.25	1.00d	1.00d	1.00	2.00	966
None inf×inoc 30 DAS×KKU25	39.88b	78.63	79.25	63.63	48.13	3.00b	3.00c	4.50	5.00	460
None inf×inoc 15 DAS×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	737
None inf×inoc 15 DAS×IT81D-1228-14-1	0.00a	0.00	0.00	1.00	2.00	1.00d	1.00d	1.00	2.00	884
None inf×inoc 15 DAS×KKU25	59.50a	29.13	52.63	52.88	46.00	5.00a	5.00a	5.00	5.00	631
Inf 15 DBS×non ino×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	916
Inf 15 DBS×non ino×IT81D-1228-14-1	0.00a	0.00	0.00	1.00	1.63	1.00d	1.00d	1.00	2.00	1009
Inf 15 DBS×non ino×KKU25	5.00cd	11.75	13.75	14.38	42.13	3.25b	3.75b	4.00	5.00	732
Inf 15 DBS×inoc 30 DAS×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	691
Inf 15 DBS×inoc 30 DAS×IT81D-1228-14-1	0.00a	0.00	0.00	1.13	1.88	1.00d	1.00d	1.00	2.00	955
Inf 15 DBS×inoc 30 DAS×KKU25	60.88a	69.00	75.63	76.38	90.25	3.00b	3.50bc	4.75	5.00	550
Inf 15 DBS×inoc 15 DAS×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	698
Inf 15 DBS×inoc 15 DAS×IT81D-1228-14-1	0.00a	0.00	0.00	1.00	2.13	1.00d	1.00d	1.00	2.00	960
Inf 15 DBS×inoc 15 DAS×KKU25	49.25ab	44.63	59.60	42.50	86.13	5.00a	4.75a	5.00	5.00	526
Inf 30 DBS×non ino×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	631
Inf 30 DBS×non ino×IT81D-1228-14-1	0.00a	0.00	0.00	1.50	1.75	1.00d	1.00d	1.00	2.00	622
Inf 30 DBS×non ino×KKU25	8.75c	10.00	15.63	21.50	40.00	4.75a	5.00a	5.00	5.00	592
Inf 30 DBS×inoc 30 DAS×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	634
Inf 30 DBS×inoc 30 DAS×IT81D-1228-14-1	0.00a	0.00	0.00	1.38	2.50	1.00d	1.00d	1.00	2.00	1000
Inf 30 DBS×inoc 30 DAS×KKU25	10.38c	76.50	66.25	81.75	84.75	5.00a	4.00b	4.75	5.00	535
Inf 30 DBS×inoc 15 DAS×KVC7	0.00d	0.00	0.00	0.00	0.00	1.00d	1.00d	1.00	1.00	735
Inf 30 DBS×inoc 15 DAS×IT81D-1228-14-1	0.00d	0.00	0.00	1.25	2.00	1.00d	1.00d	1.00	2.00	638
Inf 30 DBS×inoc15 DAS×KKU25	52.50ab	24.75	46.63	51.25	100.00	5.00a	5.00a	5.00	5.00	479
F-test	**	NS	NS	NS	NS	**	**	NS	NS	NS
CV (%)	35.71	36.31	31.14	24.61	32.57	11.50	17.67	14.30	3.62	26.02

Letter (s) within column indicate Least Significant Differences (LSD) of Duncan's Multiple Range Test (DMRT) at probability of (p) **0.01, DAS: Days After Sowing of seeds in main plots, DBS: Days Before Sowing of seeds in main plots, DAS: Days After Sowing of seeds in main plots, Inf: Infector rows, Inc: Inoculation, NS: None Significant, CV: Percentage of covariance

Interactions among contributed factors: With an interaction among the three factors, i.e., A×B×C, the results showed that a severe disease incidence was found with KKU25 where the percentage of infection was higher for inoculation at day 15 after sowing than at day 30 after sowing with values of 60.88 and 52.50%, respectively (Table 5). There were no differences between days 15 and 30 but the differences among the cultivars were relatively large and highly significant. There were no significant differences found with all subsequent sampling periods until day 65 after sowing. A severe infection (KKU25) was much greater with infector rows than none infector rows and the differences were large and highly significant. The results on severity levels indicated that at day 37 after sowing, KKU25 had much higher disease infection than the rest. This was found in all treated plants but the plots with infector rows attained much higher values than none infector rows and the differences were large and highly significant. Inoculated at 15 days after sowing gave much higher infection than inoculated at 30 days after sowing. A similar trend due to the amount of infection was also attained with the results on day 44 after sowing. However, there were no statistical differences found with the subsequent sampling periods of 51 and 58 days after sowing.

DISCUSSION

It was found with the previous study of Sinsiri and Laohasiriwong (2008) that the most appropriate rate of pathogenic spores of the disease (Pseudocercospora Leaf Spot) to be inoculated for the trial was a 5×10^4 of disease forming units mL^{-1} of distilled water and the cowpea cultivars of KVC7 and IT81D-1228-14-1 were of immune and high resistant properties against the disease, respectively whilst KKU25 cultivar was the most susceptible cultivar. Thus this rate of pathogenic disease was used for this current investigation and both cultivars were used again as to compare the results between the plots with and without infector rows on disease incidences and severity of infection so that the attained results could possibly be more reliable. It was found that effects on infector rows and inoculated dates on disease incidences (%) were not found from day 37 up to day 58 after sowing of seeds in main plots but significantly found at day 65 where the plots of infector rows of both dates (15 and 30 days before sowing of seeds in main plots) were similar but infected incidence percentages were significantly higher than the plots without infector row. The results agree with the study reported by

Nakawuka and Adipala (1997) and Adejumo *et al.* (2001). The results also indicated some advantages in applying infector rows on intensity of disease incidences and severity level significantly higher than without and a severity level was significantly higher for 15 days than 30 days before sowing of seeds in main plots. The result on severity level indicated that the earlier the inoculation period (i.e., 15 days before sowing of seeds in main plots) the higher the severity level. At days 51 and 58 after sowing, there were no significant differences due to treatments found, i.e., severity levels of all treated plants were similar. This must be attributable to the disease in plots without infector row spread out most rapidly with time hence the amount of infection of all treated plants became similar and soon most of them died off due to high severity of the disease.

For the effect due to none inoculation and inoculated at 15 and 30 days after the sowing of seeds in main plots (Factor B), the results showed that inoculated at 15 and 30 days gave a similar disease incidence % but inoculated at 15 days gave highest severity level, whilst inoculated at 30 days gave a similar severity level as none inoculation. The results indicated that the cowpea plants exposed to disease earlier produced more damages than those receiving pathogenic disease later. Therefore, in order to attain a quicker outstanding results then inoculated at 15 days after sowing of seeds in main plots should of more advantages. This confirms the research reported by Fery and Dukes (2002), McCallum and Tekauz (2002) and Reid *et al.* (2002). Seed yields attained were similar yet there was a tendency that the higher the severity the lower the seed yield even though the differences found were not statistically significant.

With the effect due to cultivars (Factor C), the results revealed that KKU25 gave the highest disease incidence, whilst KVC7 and IT81D-1228-14-1 cultivars were similar. This trend was also found with severity level. The results vividly showed that there should always be some differences found among cultivars in manifesting themselves against diseases, i.e., KKU25 is the weakest cultivar, whilst KVC7 and IT81D-1228-14-1 gave a similar resistance to the disease (Sinsiri *et al.*, 2006).

With the results on B×C interactions, the results showed that disease incidences of all sampling periods from day 37 up to day 65 were severely found with KKU25 cultivar with both inoculation dates (15 and 30 days after sowing of seeds in main plots), whilst the rest were relatively small, particularly the none inoculated treatments. The results indicated that in order to justify resistant properties of the cultivars more quickly then inoculation of pathogenic disease must be introduced. A clearer result was found with severity level, i.e. inoculated at 15 days after sowing of seeds in main plots gave the highest severity level. Therefore, the best inoculation date should be carried out at day 15 after sowing of seeds in main plots.

For the results on A×B×C interactions, the results showed that interactions among the three factors on disease incidences were found only with the plants being sown at day 37 after sowing of seeds in main plots where disease incidences were relatively small for those without infector rows but largely found with those of infector rows, particularly with KKU25 cultivar. The results implied that infector rows with inoculation are always needed whenever a trial for disease determination is to be carried out. A similar result on severity levels of the disease was also attained, i.e., with the use of infector rows and inoculation, the plants of KKU25 were severely infested by pathogenic organisms where the differences were highly significant, thus the plants of weakest property could always possess its high amount of infestation (KKU25), particularly at 15 days before sowing of seeds in main plots. Seed yields were ranging from 526 to 1,009 kg ha^{-1} and there were no statistical differences found among the treated plants. This must be attributable to a severe infection of the disease damaged to all treated plants hence poor seed yields were attained. Therefore, to achieve a successful result, the use of infector rows 15 days before sowing of seeds with inoculation at 15 days after sowing of seeds in main plots should be of significant practices.

CONCLUSIONS

This research was carried out to evaluate the spread out of the pathogenic disease of *Pseudocercospora cruenta* (Sacc.) Deighton with the use of infector rows, without infector row and inoculated at 15 and 30 days after the sowing of seeds of cowpea cultivars in main plots (KVC7, IT81D-1228-14-1 and KKU25). It was found that a highly significant result on disease assessment of cowpea plants was achieved with the use of infector rows hence infector rows are needed whenever trials on evaluation of disease are to be carried out. KKU25 cultivar was severely affected by the disease, whilst KVC7 and IT81D-1228-14-1 cultivars have proven to show a high resistance to the disease. Infector rows being established 15 days ahead of the sowing of seeds in main plots gave the best results in evaluating amount of the infected disease, thus infector rows are required whenever trials on disease evaluation are taken place.

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