

Extreme variability modelling of overdispersed germination count experiments

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ABSTRACT

Germination tests are carried out for a wide variety of purposes in weed control. The variability in seed germination counts raises the overdispersion problem. The objective of this study is to compare different approaches used in solving overdispersion and to offer practical solutions to researchers. The data sets were created from seed germination counts, which examined the allelopathic effect of white cabbage (*Brassica oleracea* L. var. *capitata* L.) on the germination of some culture and weed seeds. Methanol and aqueous concentrations (30%, 40%, 50%) of dry and fresh white cabbage were used. Assuming the Poisson distribution in the generalized linear mixed model, overdispersion problem was determined in redroot pigweed (*Amaranthus retroflexus* L.), lamb's quarters (*Chenopodium album* L.) and sugar beet (*Beta vulgaris* L.) Equidispersion was determined in corn (*Zea mays* L.) and it was perfectly adapted to the Poisson distribution. In order to overcome the overdispersion problem, generalized Poisson in weeds, fresh cabbage methanol and aqueous applications were very effective reducing germination (p < 0.05). The best results in weed seeds were obtained at 50%. Unlike weeds, 30% concentration of dry cabbage methanol and aqueous were considered as the upper limit in order not to adversely affect germination in *Z. mays* and *B. vulgaris*. Consequently, in germination tests, the problem of overdispersion is inevitable as a result of excessive variability. For germination count data, generalized Poisson distribution is viable option and powerful alternative to accurately describe mean-variance relationship.

Key words: Generalized linear mixed model, germination count data, overdispersion.

INTRODUCTION

There are many germination studies that answer questions about the use of plants with allelopathic effects in weed control. It is thought that these studies can provide objective criteria to help decision-making in the management and control of weeds. By using plants with allelopathic effect, the germination of some seeds is affected negatively, while it can positively affect some seeds (Kural and Yergin, 2020). The resulting seed germination is usually presented as percentages or counts and evaluated with different statistical approaches (Michelon et al., 2019). However, it is important to comply with the assumptions of statistical methods applied to both count and percentage observations. The use of the correct statistical methods will determine whether the true relationships in the population represented in the study are well reflected by the experimental design (Gianinetti, 2020). Parametric approaches, such as ANOVA, cannot be used because the assumptions of normal distribution and homogeneity cannot be provided in experimental data produced from count data in agricultural sciences. In addition, the shape of the distribution of the data is excessively skewed to the right, due to a wide range of variation among the observations obtained by counting. In this case, various transformation methods applied (e.g., logarithmic or square root transformation) are insufficient to meet the assumption of normality (Gbur et al., 2012; Sileshi, 2012). Therefore, non-parametric methods are widely used in seed germination in plant ecology and

management. These methods assume that the count data is not normally distributed and mathematically has Poisson distribution. However, the most prominent feature of this distribution, and also a strict limitation, is that the response variable consisting of germination counts should be equal to the variance of the mean. This assumption is difficult to achieve in practice. The variance of the count-based dependent variable increases depending on the widening of the count range (excess variability or heterogeneity), and the variance is found to be greater than the mean (Kosma et al., 2019; Gianinetti, 2020). By ignoring overdispersion and using any statistical analysis method, standard errors will result in smaller than expected, inflated test statistics (e.g., F value) and rather large Type I error. This leads to important parameter estimation results being insignificant and vice versa (Sileshi, 2012; Hilbe, 2014).

The most powerful alternative to the Poisson distribution for the solution of the overdispersion problem in the studies based on count data is the use of the negative binomial distribution. This method can eliminate overdispersion by adding a parameter called the dispersion parameter to the model. Overall, this approach is a powerful one for the overdispersion situation. However, in some cases, when trying to fix the overdispersion problem, the variance becomes smaller than the mean; thus, introducing the dispersion problem. The models proposed by various researchers for count data are not able to adapt to both overdispersion and under-dispersion. The generalized Poisson distribution can be used when there is overdispersion, under-dispersion or non-dispersion (Harris et al., 2012). The most important phenomenon in all three cases is the correct determination of the model to obtain consistent and reliable results.

In this study, the effect of the white cabbage (*Brassica oleracea* L. var. *capitata* L.) plant, which is thought to have an allelopathic effect, on culture and weeds was examined by seed germination counts. The aims were to examine the problem of overdispersion in germination count datasets, evaluate the effect of overdispersion on test statistics, and compare alternative distributions in generalized linear mixed models to solve this problem.

MATERIALS AND METHODS

Germination count database

White cabbage (*Brassica oleracea* L. var. *capitata* L.) leaves grown under farm conditions were used: fresh and dried. While the fresh cabbage leaves were used after washing with distilled water, the dried seedlings were used then dried in the shade. Aqueous and methanol extracts of white cabbage were used at three different concentrations (30%, 40% and 50%). During the germination, 50 weeds, 10 corn and 30 sugar beets seeds were used. White cabbage extract (5 mL) was added to the seeds in Petri dishes and distilled water was added to the control group at the same rate. Experiments were completed after 14 d and seed counts were made for germination. The experiment was arranged in five replicates according to the completely randomized design (CRD). In this study, the dataset was used from the source (Kural, 2018; Yilmaz, 2019).

Data analyses

Poisson distribution assumption in generalized linear mixed model (Poisson-GLIMMIX) to determine whether there is overdispersion problem or not, and then Negative Binomial distribution assumption in generalized linear mixed model (NB-GLIMMIX) and Generalized Poisson distribution assumption in generalized linear mixed models (GP-GLIMMIX), which are two strong alternatives, were created in order to eliminate the problem of overdispersion. Generally, the data analyses in this study were divided into three phases as illustrated in Figure 1. Firstly, normality and homogeneity tests were evaluated from germination counts of redroot pigweed (*Amaranthus retroflexus* L.), lamb's quarters (*Chenopodium album* L.), sugar beet (*Beta vulgaris* L.) and corn (*Zea mays* L.) plants were considered to be response variables.

Then, data were analyzed with a generalized linear mixed model using the proc GLIMMIX procedure in SAS ver. 9.4 (SAS Institute, Cary, North Carolina, USA) accounting for the Poisson, negative binomial and generalized Poisson distribution of the data and using log link function. At this stage, Pearson chi-square/df goodness-of-fit statistic was used to evaluate the mean-variance relationship in the Poisson assumption of response variables in GLIMMIX. Pearson chi-square/df > 1 indicates overdispersion, Pearson chi-square/df = 1 is equidispersion, and Pearson chi-square/df < 1 indicates under-dispersion (Hilbe, 2014). After the overdispersion problem was determined, two alternative methods of NB-GLIMMIX and GP-GLIMMIX were applied. Akaike's information criterion (AIC) and Bayesian information



Figure 1. The proposed of designed framework for germination seed count analysis.

criterion (BIC) were considered to determine which model should be used in reporting the results. Fresh white cabbage methanol (FCM), fresh white cabbage aqueous (FCA), dry white cabbage methanol (DCM) and dry white cabbage aqueous (DCA) treatments and concentration levels (30%, 40% and 50%) were included as fixed effects in all models. The Bonferroni post-hoc test was used to determine the differences between fixed effects.

RESULTS

Pre-examination for overdispersion in the germination counts of plant species

For a preliminary investigation of the overdispersion problem, the mean and variance were calculated from simple baseline statistics from the germination count data sets of both weed and cultured species (Figure 2). The variation in germination counts in *A. retroflexus* and *C. album* weeds is quite large. Compared to weeds, this difference was less in *B. vulgaris* and both statistics were almost equal in *Z. mays*. Therefore, in the mean and variance plot the overdispersion problem was evident in two weeds and *B. vulgaris*. However, even distribution was thought to occur in *Z. mays*.



Figure 2. Comparison of mean and variance values of germination counts of plants.

A. retroflexus: Amaranthus retroflexus; C. album: Chenopodium album; B. vulgaris: Beta vulgaris; Z. mays: Zea mays.

In the second phase of the preliminary examination, both normality and homogeneity tests were performed separately on the plant datasets (Table 1). In the applied tests, the germination counts of the plants did not have normal distribution (p < 0.0001) and heterogeneous variance was identified (p < 0.01; p < 0.0001). This is an expected result in both count data and the overdispersion problem. In general, according to the results of the preliminary examination, the transformation (arc sine or square root) used by most researchers in count data will not provide a solution. Therefore, complex non-parametric statistical methods should be used.

Model selection for germination data

The results of the dispersion statistics and goodness of fit criteria obtained from the Poisson-GLIMMIX, NB-GLIMMIX and GP-GLIMMIX models are given in Table 2. The dispersion statistics obtained from the germination counts of *A*. *retroflexus*, *C*. *album* and *B*. *vulgaris* were determined as 5.25, 4.68 and 2.71, respectively, and were accepted as strong indicators of overdispersion (Pearson chi-square/df > 1). The propagation parameter in *Z*. *mays* was determined as 1.06 and since it was close to 1, it was accepted that there was no dispersion or it had equal dispersion. Therefore, *Z*. *mays* germination counts were considered to be compatible with the Poisson distribution.

In the next stage, we obtained interesting results from the alternative models of NB-GLIMMIX and GP-GLIMMIX, which were applied to eliminate the overdispersion situation. For example, for *A. retroflexus*, where the overdispersion statistic was higher, the dispersion criterion changed from 5.25 to 0.86 with NB-GLIMMIX, while it changed from 4.68 to 1.09 with GP-GLIMMIX. The dispersion statistic obtained from this model was closer to 1.

In this table, Akaike's information criterion (AIC) and Bayesian information criterion (BIC) were also obtained in order to determine the most suitable model for the germination counts of each plant. Since the smallest AIC and BIC values for *A. retroflexus*, *C. album* and *B. vulgaris* plants were obtained from GP-GLIMMIX, it was determined as the appropriate model. The smallest AIC and BIC values for *Z. mays* were obtained from Poisson-GLIMMIX. Parallel results were obtained from both Pearson chi-square/df and AIC and BIC measures.

Table 1. Probability levels and statistics for homogeneity of variance and test of normality in germination seed data.

Plants	Shapiro-Wilk tes	t of normality	Levene's test of homogeneity		
	Statistics	р	F	р	
Amaranthus retroflexus	0.868	0.000	3.203	0.001	
Chenopodium album	0.854	0.000	3.058	0.001	
Zea mays	0.755	0.000	4.996	0.000	
Beta vulgaris	0.868	0.000	3.816	0.000	

THE ATTEMENT OF A THE AUDIT OF A AND A AND A AND AND AND AND AND AND A	Table 2. Results of overd	ispersion parameters and	l goodness of fit criteri	a for all three	regression models
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	Poissor	n-GLIMMIX	NB-0	GLIMMIX	GP-GLIMMIX		
Plants	Pearson	Pearson Chi-Square/df	Pearson	Pearson Chi-Square/df	Pearson	Pearson Chi-Square/df	
A. retroflexus	383.57	5.25	62.52	0.86	79.87	1.09	
C. album	341.58	4.68	66.52	0.91	71.49	0.98	
Z. mays	77.03	1.06	80.22	1.10	76.50	1.05	
B. vulgaris	197.78	2.71	70.91	0.97	76.64	1.05	
	AIC	BIC	AIC	BIC	AIC	BIC	
A. retroflexus	786.34	803.02	576.40	595.46	573.72	592.78	
C. album	721.73	738.40	560.98	580.04	552.65	571.71	
Z. mays	374.43	391.11	377.60	396.66	376.43	395.49	
B. vulgaris	559.28	575.96	507.20	526.25	505.50	524.56	

Poisson-GLIMMIX: Poisson distribution assumption in generalized linear mixed model; NB-GLIMMIX: Negative Binomial distribution assumption in generalized linear mixed model; GP-GLIMMIX: Generalized Poisson distribution assumption in generalized linear mixed model models; AIC: Akaike's information criteria; BIC: Bayesian information criteria (AIC or BIC: small is better); *A. retroflexus: Amaranthus retroflexus; C. album: Chenopodium album; B. vulgaris: Beta vulgaris; Z. mays: Zea mays.*

The results for the fixed effects from Poisson-GLIMMIX, NB-GLIMMIX and GP-GLIMMIX are given in Table 3. When the test statistics for overdispersed germination seed counts of Poisson-GLIMMIX for *A. retroflexus*, *C. album* and *B. vulgaris* plants were examined, the F values were quite high and the p values were equally small, for both treatments and concentrations.

However, the decrease in F values obtained from applications and concentrations in the alternative models of NB-GLIMMIX and GP-GLIMMIX models was remarkable. The F values obtained from GP-GLIMMIX, which was determined as the appropriate model for all three plants, were smaller than NB-GLIMMIX. The effects of treatments and concentrations on germination were found to be significant for both weeds and cultured plants (p < 0.0001).

The least squares means, standard errors and Bonferroni multiple comparison results for determining the differences between treatments and concentrations in germination seed counts of plants are shown in Figures 3 and 4, respectively.

In the treatment results in Figure 3, the differences in standard errors are most evident in the model results for *A*. *retroflexus*, *C*. *album* and *B*. *vulgaris*. Standard errors in Poisson-GLIMMIX for these three plant counts with overdispersion are much smaller than expected compared to the other models. In Bonferroni test results to determine the differences between applications, fresh white cabbage methanol (FCM) and fresh white cabbage aqueous (FCA) applications were more effective than dry white cabbage methanol (DCM) and dry white cabbage aqueous (DCA) in reducing weed seed germination (p < 0.05).

Contrary to treatments, Figure 4 suggests that the concentrations applied for plant germination were more effective in the occurrence of overdispersion. Standard errors at all concentrations for *A. retroflexus* and *C. album* with NB-GLIMMIX and GP-GLIMMIX are close to each other.

Differences in lettering according to Bonferroni test results for Poisson-GLIMMIX, NB-GLIMMIX and GP-GLIMMIX models also revealed the importance of determining the appropriate model for the datasets. For example, the effects of control and all concentrations in Poisson-GLIMMIX were significant (p < 0.05) for weeds with high overdispersion. However, the effect of all concentrations on germination was significant (p < 0.05) in NB-GLIMMIX and GP-GLIMMIX compared to the control. In both models, both weeds and seeds of *B. vulgaris* had concentration levels of 30% and 40%. The effects of 40% and 50% concentrations on germination were similar. *Zea mays*, where there was no overdispersion, the standard errors increased as the concentrations increased compared to the control level in all models. There was a difference between the concentration levels compared to the control, but the effects of the three concentrations on germination were almost similar.

Generally, when the application and concentration results are evaluated together (Figures 2 and 3), while 50% concentration level of FCM and FCA applications of white cabbage prevented seed germination in weeds, DCM and DCA applications increased germination in cultured plants, but as the concentration levels increased, there was a decrease in germination rates.

	Poisson-GLIMMIX				NB-GLIMMIX				GP-GLIMMIX				
Plants	Tr	Treatment		Concentration		Treatment		Concentration		Treatment		Concentration	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	
A. retroflexus	101.3	< 0.0001	196.0	< 0.0001	40.0	< 0.0001	45.1	< 0.0001	22.4	< 0.0001	21.8	< 0.0001	
C. album	72.8	< 0.0001	234.0	< 0.0001	34.4	< 0.0001	58.5	< 0.0001	17.4	< 0.0001	38.5	< 0.0001	
Z. mays	28.1	< 0.0001	14.2	< 0.0001	22.9	< 0.0001	9.0	< 0.0001	26.8	< 0.0001	13.9	< 0.0001	
B. vulgaris	81.5	< 0.0001	80.3	< 0.0001	39.7	< 0.0001	34.7	< 0.0001	29.8	< 0.0001	23.5	< 0.0001	

Table :	3.	Results	of	the	fixed	effects	from	the	models.

Poisson-GLIMMIX: Poisson distribution assumption in generalized linear mixed model; NB-GLIMMIX: Negative Binomial distribution assumption in generalized linear mixed model; GP-GLIMMIX: Generalized Poisson distribution assumption in generalized linear mixed model models; *A. retroflexus: Amaranthus retroflexus; C. album: Chenopodium album; B. vulgaris: Beta vulgaris; Z. mays: Zea mays.*



Figure 3. GLIMMIX models containing least squares means and comparisons for treatments.

Poisson-GLIMMIX NB-GLIMMIX GP-GLIMMIX

Different letters in the bars indicate significant differences between treatments according to the Bonferroni test (P < 0.05). Vertical bars correspond to standard error.

Ls Mean: Least square means; SE: standard error; DCM: dry white cabbage methanol; DCA: dry white cabbage; FCM: fresh white cabbage methanol; FCA: fresh white cabbage aqueous; *A. retroflexus: Amaranthus retroflexus; C. album: Chenopodium album; B. vulgaris: Beta vulgaris; Z. mays: Zea mays*; Poisson-GLIMMIX: Poisson distribution assumption in generalized linear mixed model; NB-GLIMMIX: Negative Binomial distribution assumption in generalized linear mixed model; GP-GLIMMIX: Generalized Poisson distribution assumption in generalized linear mixed model.



Figure 4. GLIMMIX models containing least squares means and comparisons for concentrations.

Different letters in the bars indicate significant differences between concentration according to the Bonferroni test (P < 0.05). Vertical bars correspond to standard error.

Ls Mean: Least square means; SE: standard error; A. retroflexus: Amaranthus retroflexus; C. album: Chenopodium album; B. vulgaris: Beta vulgaris; Z. mays: Zea mays; Poisson-GLIMMIX: Poisson distribution assumption in generalized linear mixed model; NB-GLIMMIX: Negative Binomial distribution assumption in generalized linear mixed model; GP-GLIMMIX: Generalized Poisson distribution assumption in generalized linear mixed model.

DISCUSSION

Over or under-dispersion is not an uncommon phenomenon in count data from agricultural trials. Therefore, this study was organized for two purposes. The first was the evaluation of the dispersion criterion, which is frequently used in GLIMMIX to determine the overdispersion status in small samples. Second, the generalized Poisson distribution was used as an alternative model to the negative binomial distribution used in the analysis of overdispersion.

In the first stage of the study, a high level of overdispersion was determined in weeds with higher variation of seed germination counts (Pearson chi-square/df = 4.68-5.25 range). Although there was less variation in germination counts of *B. vulgaris* among cultured plants compared to weeds, the problem of overdispersion emerged (Pearson chi-square/df = 2.71). Germination counts of *Z. mays* were determined not to have overdispersion (Pearson chi-square/df = 1.06). Overdispersion is accepted as 1.0 < Pearson chi-square/df < 1.0 dispersion status in most studies (Kosma et al., 2019; Michelon et al., 2019; Prazaru et al., 2021) as a general approach to determine over, dispersion or perfect fit to Poisson distribution. However, there is no official criterion for this. Gbur et al. (2012) suggested that Pearson chi-square/df values greater than two, which they recommend as a very general guideline based on experience, are a potential indicator of overdispersion. In our results, significant differences were observed between the results of Poisson and alternative models for weeds germination where the Pearson-chi square/df statistic was greater than 2. The same situation occurred in the germination of *B. vulgaris*, where the dispersion statistic was smaller than the weeds (Pearson chi-square/df = 2.70).

(Pearson chi-square/df = 2.70). However, Figure 3 shows the effect of this overdispersion in *B. vulgaris* DCM and DCA applications. While the difference between DCM and DCA applications in Poisson-GLIMMIX and NB-GLIMMIX was significant (p < 0.05), there was no difference between both applications in GP-GLIMMIX, which was determined as the most suitable model for this plant (p > 0.05). Considering these results was suggested that the measure of dispersion should be greater than two (Pearson chi-square/df ≥ 2) to determine overdispersion in germination counts of plants. In the study, in which two alternative methods NB-GLIMMIX and GP-GLIMMIX were used to solve the problem of overdispersion, both AIC and BIC fit criteria were used to determine which model results should be reported in datasets containing the same observations, the same explanatory variables and germination counts of the same plants. In weed results, the dispersion statistics were obtained from the two alternative models, with GP-GLIMMIX results being closer to 1. Contrary to expectations, values smaller than 1 were obtained for NB-GLIMMIX. Because of this, Gbur et al. (2012) showed that the extra dispersion parameter added to the model to correct for overdispersion in negative binomial distribution tends to estimate the extent of overdispersion in the data in small samples where the degree of overdispersion is less. Similarly, Sellers and Morris (2017) stated it may exhibit the behavior of being smaller when estimating the dispersion statistic, since the dispersion parameter in NB-GLIMMIX is more variable than in GP-GLIMMIX.

The smallest AIC and BIC were obtained from the GP-GLIMMIX model as the appropriate model in the germination counts of *A. retroflexus* and *C. album*. In generalized linear mixed model, both diffusion measures and AIC and BIC measures act parallel to each other. However, Gbur et al. (2012), Michelon et al. (2019) and Kosma et al. (2019) showed that although AIC and BIC criteria are effective in model selection, a decision should be made by examining Pearson chi-square/df since they cannot distinguish over or under dispersion in Poisson distribution. The results obtained from the culture plant *B. vulgaris* support this situation because the dispersion statistics for NB-GLIMMIX and GP-GLIMMIX were obtained as 0.97 and 1.05. In both models, the problem of excessive dispersion was eliminated and the closest value to one was obtained from NB-GLIMMIX. However, in the selection of the model suitable for the dataset, the smallest AIC and BIC were obtained with the GP-GLIMMIX model.

Zea mays, on the other hand, perfectly fitted the Poisson distribution, as the variance and mean were close to each other. In this case, interesting results were obtained from Poisson-GLIMMIX (Pearson chi-square/df = 1.06), NB-GLIMMIX (Pearson chi-square/df = 1.01) and GP-GLIMMIX (Pearson chi-square/df = 1.05) with outcomes close to each other. Saputro et al. (2021) stated the reason for getting close results from these distributions is that the mean of all three probability distributions is almost equal to their variance in the absence of dispersion (dispersion parameter = 0). Because these distributions are derived from each other, they use the same link function (log link) when modeling germination counts with application and concentrations in the GLIMMIX model. Therefore, the results obtained from all three models are similar to each other.

In our results, the effects of application and concentrations included in the models as explanatory variables for germination were significant, while the standard errors obtained from Poisson-GLIMMIX were very small in overdispersed plant data. If we were to focus on the Poisson distribution hypothetically without examining the overdispersion situation, the standard errors of the concentrations or doses would be very small (underestimated) and the test statistics would be very large, so the results that were important would be insignificant or the opposite (Kosma et al., 2019; Michelon et al., 2019).

In weeds, in both NB-GLIMMIX and GP-GLIMMIX results, FCM and FCA applications were very effective in reducing weed germination, in parallel with the increase in concentration levels. In these applications, the highest concentration level of 50% was very effective on the germination of weeds. It was determined that DCM and DCA applications were less effective on germination in contrast to FCM and FCA applications. Unlike weeds, DCM and DCA applications are more effective in germination in the results of *Z. mays* in Poisson-GLIMMIX and *B. vulgaris* in GP-GLIMMIX. Compared to the control, as the concentration levels increased, there was a decrease in germination rates especially for *B. vulgaris*, but no serious effect was detected in *Z. mays*. In general, lower concentrations should be preferred in order to not adversely affect germination in both cultivars, and concentration levels of 30%-40% can be considered as the upper limit. In the results, there was no negative effect on seed germination of cultured plants, while white cabbage plants significantly reduced weed germination.

CONCLUSIONS

The generalized Poisson distribution (GP-GLIMMIX) provided consistent and precise parameter estimates (smaller standard error) compared to the negative binomial distribution (NB-GLIMMIX). GP-GLIMMIX has been proposed as a stronger alternative to NB-GLIMMIX in solving the common problem of excessive variability or overdispersion problem in seed germination counts.

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