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Investigation of factors influencing seed load analysis results

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Master thesis, chemistry

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INTRODUCTION

Chemical seed treatments are widely used in agriculture to manage early-season insect infestations and diseases, as well as seedborne pathogens that attack seed, and seedlings [1],[2]. Different equipment and materials are utilized to achieve different seed treatments (Fig. 1). Seed load analysis by HPLC is an element of quality assurance to monitor the treatment process [2]. The large number of existing methods, some of which provide different results for the same samples, raises concerns about the validity and usefulness of these analyses. Fig. 2 shows possible causes that may influence the loading results. The aim of this work is to determine the influencing factors on the seed load analysis results and thus to explore the possibility for the feasibility of a set of global methods and to show possible approaches to realise this.

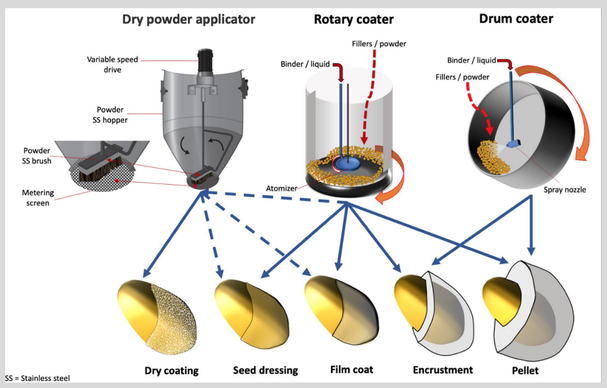

Fig. 1: Important seed treatment equiupment types and methods [1].

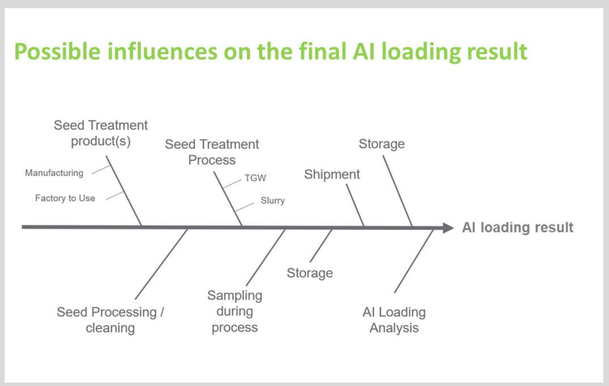

Fig. 2: Possible factors influencing the seed load analysis result.

CONCEPT

For the work, 14 active ingredients (AI) and 4 crops plus a surrogate matrix (glass beads) were prioritized. A UPLC-method for the quantification of the AI was developed and validated followed by the development of a model treatment (Fig. 3), which enabled the treatment of the crops on a laboratory scale under mass balance conditions. A set of possible influencing factors was defined and investigated by means of design of experiment. The data obtained was evaluated with a semi-automatic process (Fig. 4) and translated into a method for analyzing the AI whose application risk was assessed for each AI and suggestions for minimizing it were made. From all the findings and results of this work, a standardized approach was developed for the investigation of further or new AI.

Fig. 3: Developed conical flask treatment method.

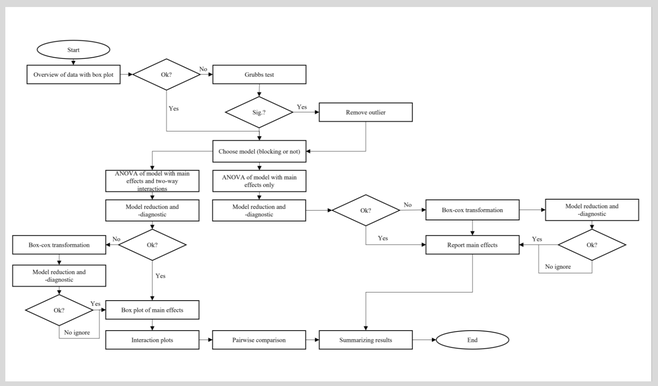

Fig. 4: Flowchart of semi-automatic data evaluation.

RESULTS

Fig. 5: Results of treatment according to the developed conical flask treatment method.

Table 1: Risk assessment for the application of the proposed global method and the estimated marginal mean recoveries with the corresponding confidence intervals.

Legend

No risk
Low risk
Medium risk
High risk

Prio. 1 AI [a]	Soybean (n=9)	OSR (n=7)	Corn (n=9)	Wheat (n=8)	Control (n=9)
CYNT	99.5 ± 2.8	98.3 ± 2.3	99.9 ± 3.5	98.3 ± 2.9	-
FDL	97.0 ± 2.9	95.0 ± 2.0	96.8 ± 3.4	96.1 ± 2.6	98.5 ± 3.4
SDX	97.3 ± 3.3	95.4 ± 3.1	96.0 ± 2.7	97.4 ± 4.3	102 ± 3.2
TYM	99.6 ± 3.7	99.4 ± 3.2	100 ± 2.8	98.1 ± 3.6	-
TFT	95.2 ± 4.5	96.8 ± 2.8	97.8 ± 2.9	95.7 ± 4.9	98.9 ± 3.9
TMX	95.9 ± 3.7	89.3 ± 5.3	85.8 ± 4.0	90.9 ± 2.4	101 ± 3.6
MFX	89.4 ± 4.2	82.1 ± 5.6	77.3 ± 4.4	84.8 ± 5.3	101 ± 4.7
APN	99.1 ± 3.5	99.2 ± 4.0	99.4 ± 3.1	99.6 ± 3.9	100 ± 2.5
Prio. 2 AI [b]	**Soybean (n=4)**	**OSR (n=4) [c]**	**Corn (n=4)**	**Wheat (n=4)**	**Control (n=5)**
TBZ	99.1 ± 2.3	94.7 ± 5.6	96.1 ± 4.8	95.7 ± 5.8	102 ± 3.9
ASM	95.4 ± 11.7	91.2 ± 8.5	87.2 ± 12.8	82.7 ± 18.2	56.2 ± 42
AZ	98.5 ± 7.5	96.3 ± 4.4	98.9 ± 6.1	96.2 ± 8.2	101 ± 4.7
OXTP	101 ± 1.9	97.4 ± 6.0	100 ± 6.8	97.1 ± 6.7	102 ± 4.3
PCBX	98.8 ± 3.5	96.3 ± 8.0	99.6 ± 4.8	96.0 ± 7.3	101 ± 2.7
PLN	101 ± 4.1	98.4 ± 5.7	102 ± 6.9	97.9 ± 7.0	101 ± 6.0

[a] Estimation of marginal mean recovery for each prio. 1 AI per crop and the corresponding confidence interval (α = 5%). Averaged over all factor levels except level MeOH and 50% water of the factor solvent and additional water during extraction.
[b] Estimation of marginal mean recovery for each prio. 2 AI per crop and the corresponding confidence interval (α = 5%). Averaged over all factor levels except level MeOH and 50% water of the factor's solvent and additional water during extraction
[c] Estimated marginal mean recovery for OSR of all prio. 2 AI includes only two levels of factor storage time, namely 0 and 14 days.

Table 2: Reassessed risk for TMX and MFX using separate methods and the estimated marginal mean recoveries with the corresponding confidence intervals.

AI	Soybean (n=9)	OSR (n=7)	Corn (n=9)	Wheat (n=9)	Control (n=8)
TMX [a]	89.3 ± 2.8	97.7 ± 5.6	94.9 ± 2.4	94.5 ± 3.5	98.2 ± 3.1
MFX [b]	94.6 ± 3.5	97.3 ± 3.4	96.4 ± 3.9	96.2 ± 3.1	99.0 ± 2.0

[a] Estimation of marginal mean recovery for TMX per crop and the corresponding confidence interval (α = 5%). Averaged over all factor levels except level ACN and 0% water of the factor solvent and additional water during extraction.
[b] Estimation of marginal mean recovery for MFX per crop and the corresponding confidence interval (α = 5%). Averaged over all factor levels except level MeOH and 0% water of the factor solvent and additional water during extraction.

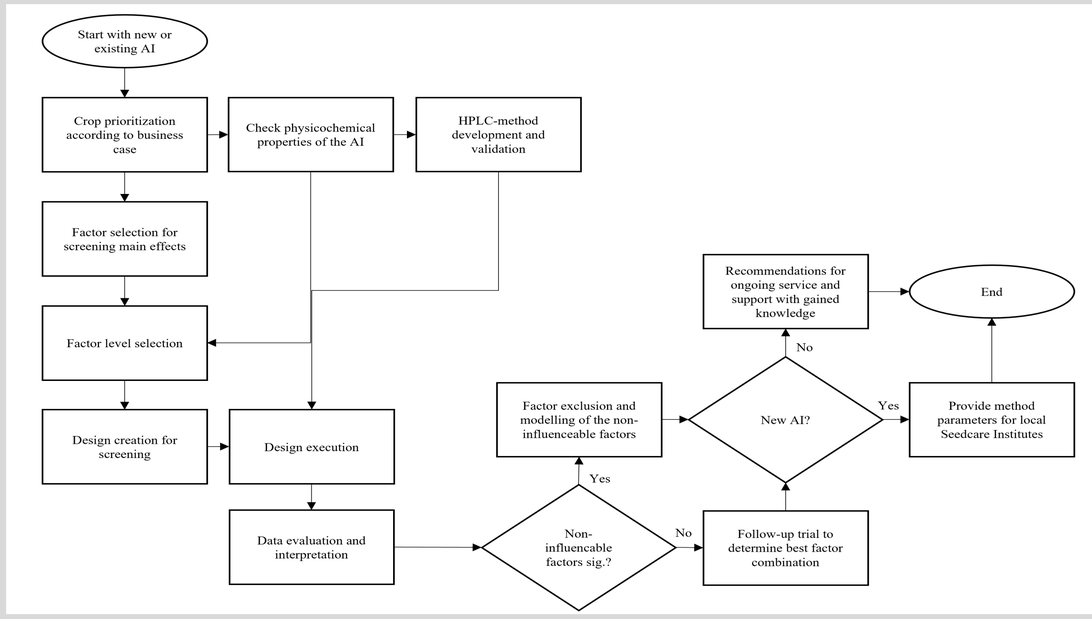

Fig. 6: Standardized approach to find appropriate seed load analyisi method.

CONCLUSION

From the data evaluation, the influencing factors could be determined, and a suitable global method could be defined, which can quantify most AI reliably, without risk. For two AI, the high risk could be reduced with a separate method for each. For the AI with a residual risk, risk-reducing follow-up trials were proposed. The developed approach is a way to investigate new or present AI and to develop reliable analysis methods.

REFERENCES

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[2] Black, M.; Bewley, J.D., 2000. Seed Technology and its Biological Basis. Sheffield i.a.: Sheffield Academic Press.