Pertanika J. Trop. Agric. Sci. 35 (S): 71 - 78 (2012)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Bioefficacy of Controlled Release Formulations of Diuron on *Brassica rapa*

Norida, M.[1]*, Omar, D.[2] and Mohamad, R. B.[3]

[1]*Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[3]*Department of Plant Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

ABSTRACT

Controlled-release formulations (CRF) of diuron were prepared in laboratory using the concept of physical matrix utilizing sodium alginate and kaolin. The release rates of diuron from the formulations were determined by chemical assay its efficacy on plant was tested on *Brassica rapa*. In the chemical assay using high performance chromatography with UV detector, significant differences in the release rate between formulations were observed from 3 to 7 days after the granules had been placed in distilled water. The formulation having a 1:1 ratio of alginate to kaolin with 1mm granule size showed the fastest release of diuron, while release from the 2mm granules was slower. Increasing the proportion of kaolin to sodium alginate in the CRF reduced the release rate of active agent. The bioefficacy using *Brassica rapa* as a bioindicator showed that CRF released slower than the conventional formulation at the beginning of the treatments. In the 3rd week after the treatment (WAT), there was no significant difference in the mortality as compared to the conventional formulation at 16 WAT. The same results were also observed up to 24 WAT, the CRF caused between 40-70% mortality, while the conventional formulation treatment caused only 6% mortality. Among the CRF, the AK-2 with 1:1 ration of alginate:kaolin was found to have given the best result with the highest percentage mortality of the seedlings.

Keywords: Sodium alginate, slow release, herbicide, bioefficacy, chemical assay

INTRODUCTION

Weeds are one of the most important pests, while herbicide is the main method used in

ARTICLE INFO

Article history:
Received: 29 May 2012
Accepted: 19 October 2012

E-mail addresses:
noridamz@putra.upm.edu.my (Norida, M.)
zolkhifli@agri.upm.edu.my (Omar, D.)
rosli@agri.upm.edu.my (Mohamad, R. B.)
*Corresponding author

ISSN: 1511-3701

controlling this particular pest. In order to achieve control of weeds, it is necessary to maintain an appropriate concentration of chemical in contact with the plants for a sufficient amount of time for absorption to take place. In order to counter environmental losses and maintain the concentration above the minimum threshold of activity, applications of excessive amounts of conventional formulation of the herbicide are generally required. Nonetheless, a disadvantage of the conventional herbicide is the immediate release of active ingredient, which will partly be lost to environment consequences from the processes such as chemical and biodegradable, photolysis, evaporation, surface run-off and percolating to groundwater (Tomlin, 2002). These loses of the pesticide affectivity could reach as high as 30% (Cespedes *et al.,* 2007). The increase in the application rate, however, is likely to increase the potential for adverse impact on the environment. One of the effective approaches to reduce environmental loss while providing more efficient weed control is through the use of controlled release formulation (CRF).

Controlled release pesticide technology has received increased attention for the last two decades due to the growing awareness that pesticides may produce undesirable environmental effects when applied in conventional formulations at the levels required for activity (Kydonieus, 1980). The controlled release pesticides are better than conventional formulation in reducing leaching (Fernandex-Perez *et al.*, 1999), volatilization (Dailey, 2004) and its prolonged activity (Bahadir, 1987). Numerous types of matrices have been tested for the preparation of CRF herbicides, while different mechanisms of release rate are often involved (Murphy & Barrett, 1990). Alginate has been used due to the unique properties they possess such as high biocompatibility and biodiegradbility (Pasparakis & Baouropoulus, 2006). It has been reported to be used in medical applications (Wan, Heng, & Chan, 1992), as well as in agriculture (Kulkarni *et al.*, 2000). For the use in herbicide it has been studied on diquat and dichlobenil (Barret & Logan, 1982), 2, 4-D (Connick, 1982), thiobencarb (Hussain *et al.,* 1982), as well as alachlor and atrazine (Gerstl, 1994). Many research on controlled release has emphasized on different sorbents and kinetic release on pesticide from controlled release formulations (Garrido-Herrera *et al*., 2006; Kumar *et al*., 2010; Flores-Cespedes *et al*., 2007); however, little is known about the efficacy of the controlled release formulation pesticide in controlling the target pest. Therefore, the objective of the study was to determine the bioefficacy and the prolong efficiency of CRF formulations of diuron on the bioindicator, *B. rapa*.

MATERIALS AND METHODS

The alginates CRF diuron was prepared based on the gelling properties of the alginate with kaolin as filler. It was made up of the formulation in water containing 20% of diuron with three different ratios of kaolin over sodium alginate. Sodium alginate of 2% viscosity and kaolin are both obtained from Sigma Chemicals. The solution was homogenized with magnetic stirrer for 1 hour. The alginate mixtures were dropwise added into 0.25 M calcium dichloride ($CaCl_2$) by Easy-Load Masterflex® peristaltic pump. Materflex Pharmed® tubing, size numbers 13 and 14, were used to give 1 and 2 mm granule size, respectively. The 1 mm granules were coded as AK-1, AK-2, AK-3 for 4:0, 1:1 and 1:3 ratio of alginate to kaolin, respectively. The 2 mm granules were coded as AK-1b, AK-2b and AK-3b for the same ratio. The beads were filtered and dried at room temperature.

Herbicide Release from CRF by Chromatographic Analysis

The release rate for diuron from formulated CRFs was analysed by reverse-phase high performance liquid chromatography. For this purpose, 0.25 g of CRF (equivalent to 0.02 g a.i) was added for each sample (five replicates) and placed in volumetric flasks containing 25ml distilled water. The flasks were capped and stored in dark environment at room temperature. Samples of 30 uL from each flask at 3, 6, 10, 15 and 20 days were taken and then injected into liquid chromatography for the quantification of diuron.

A Waters 501® liquid chromatography with Nova Pak HR C 18 reverse phase colume (3.9 x 300 mm) was operated at a flor rate of 1 ml/min using mobile phase of acetonitrile:distilled water (60:40). The UV-VIS Waters® 484 Tunable absorbance Detector set at 254 wavelengths was used to detect diuron. Each of the 20 uL samples was injected into the pump by using the 100 uL Hamilton® syringe. Peaks were recorded by the computerize Waters System Interface Module (SIM) and analyzed by the Baseline programme.

Bioefficacy of CRF on the Bioindicator Brassica rapa

The granules of CRF and wettable powder CF were applied at a mass application rate of 1 kg ai/ha. The granules of CRF were applied by spreading them evenly in the pots and the amount applied per pot was 38mg. The treatments were applied to the soil one day before sowing one hundred *Brassica rapa* per pot. The soil was watered twice daily throughout the experiment. The seeds were germinated at 5 days after sowing (DAS) and symptoms of leaf chlorosis, leaf tip burning and death of the seedlings were only observed at 8 DAS.

The percentage mortality of the seedlings was recorded at 10 and 14 DAS, followed by removing all the seedlings at 20 DAS. 100 seeds were sown again at 3 weeks after the treatment (WAT). Similar procedures and data collections as the first batch of seeds were followed. The subsequent batches of seeds were sown at 6, 8, 9, 10, 16 and 24 (WAT). The seedling mortality was assessed visually based on a qualitative scale (Burril *et al.,* 1976). The experiment was a completely randomized design (CRD) with six replications. All the data were subject to the analysis of variance and the treatments were compared using Tukey's Test.

RESULTS AND DISCUSSION

Release Rate of CRF by Chromatographic Analysis

The retention time of diuron for the chromatographic condition described earlier was 3.8 minutes. Fig.1 shows the cumulative release of diuron into water from various formulations which occurred in a controlled manner. After 3 days, the AK-2 formulation was the fastest to release the diuron. The amount detected was 16.1% of the original content of diuron in the formulation. During the same period, AK-1, AK-3, AK-1b, AK-2b and AK-3b released 11.1, 15.5, 8.10 and 10.7% of diuron, respectively. More diuron was released from all the formulations after 6 days. The increase compared to the release at 3 days was more than 100% for AK-1, AK-1b, AK-2b and AK-3b, 60% for AK-2 and 48% for AK-3. The amount of diuron released reached the maximum on day 15, as the amount recorded for 20th days is the same as that of 20th days . The release on the 20th day showed that the 1 mm granules released higher amount of diuron compared to the 2 mm granules, with the following increasing order: AK-1b<AK-2b<AK-3b<AK-3<AK-1<AK-2. The release of diuron from CRF did prolong as compared to conventional formulations pesticide. Fernandez-Perez *et al.* (1998) used conventional formulation of imidacloprid to compare it with CRF and found the technical imidacloprid was completely dissolved within 2 hours, while Garrido-Herrera *et al*. (2006) discovered that 100% technical grade of isoproturon and imidacloprid were fully dissolved within 3 days and 3 hours, respectively. As technical grade of pesticide would loss up to 5% during the preparation (Cotteril *et al*., 1996), the release of diuron from this experiment therefore did not reach the full amount of its content. The results showed a distinct effect of granule size on the rate of release. As expected, the smaller the granule diameter, the faster the rate of release. Thus, it is apparent that the use of sodium alginate

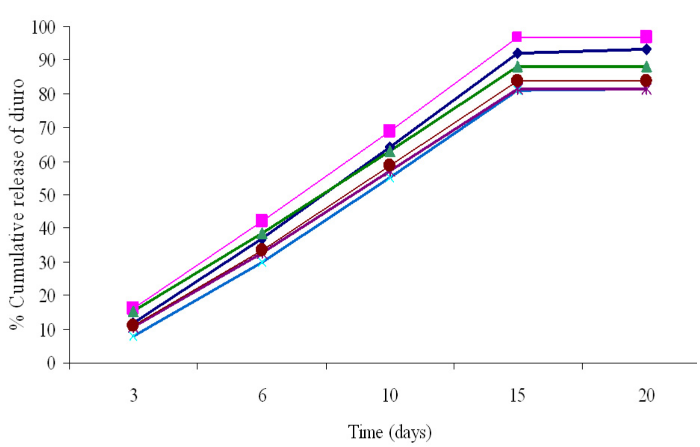

Fig.1: Cumulative release of diuron from alginate-kaolin controlled release formulation into distilled water. AK-1=◆; AK-2=■; AK-3=△ ; AK-1b=✕; AK-2b=◇; AK-3b=□

increased the crosslinking density of the formulation, while kaolin (as filler) slowed down the release of herbicide from the CRF. Similar responses have also been reported by some earlier researchers (e.g. Hussain *et al.,* 1992; Gerstl *et al.,* 1994).

Determination of CRF Release Rate by Bioassay

The percentage mortality of *Brassica rapa* seedlings exposed to CF and CRF is shown in Tables 1-3. Based on the assessment made at 10 and 14 DAS, a significantly higher mortality was observed for the CF treatment with 18.7% and 65%, respectively, as compared to other formulations after 1 week of the treatment (WAT) (see Table 1). This indicated the immediate availability of diuron from the CF. The highest mortality obtained from the CRF treatments was only 10% at 10 DAS and this was obtained from the AK-1, AK-2 and AK-2b formulations. At 14 DAS, the AK-1 formulation showed 35% mortality, which was significantly higher compared to AK-1b and AK-3b formulations, where the percentage of mortality was recorded at only 15%. This could be due to CRF released diuron slower and hence, insufficient doses were available to produce similar effects of CF. At 3 WAT (see Table 1), no significant difference was observed between the treatments based on the mortality at 10DAS, except for AK-2b. At 14DAS, there was no significant difference between all the treatments. This showed that the release of diuron from the CRF had increased, and hence, sufficient doses were released to show similar result as the CF treatment. A similar result was also obtained at 6 WAT.

At 8 WAT (Table 2), the AK-1b and AK-2b formulations showed significantly lower mortality compared with that of the CF, while other formulations indicated no significant difference with the CF. This could be due to uneven release of active ingredient from bigger granules, causing inconsistent results on the percent mortality. There was no significant difference on the mortality of the seedlings at 14 DAS for

TABLE 1
The effects of CF and CRF on the percentage mortality of *Brassica rapa* seedlings at 1, 3 and 6 weeks after the treatments (WAT)

Treatments	Percentage Kill **					
	1 WAT		3 WAT		6 WAT	
	10 DAS	14 DAS	10 DAS	14 DAS	10 DAS	14 DAS
CF	18.7±1.25 a	65±2.88 a	100±0.00 a	100±0.0 a	85.0±2.88 a	95.0±2.04 a
AK-1	10.0±0.0 b	35.0±2.88 b	90.0±3.53 a	98.7±1.25 a	85.0±2.88 a	93.7±1.25 a
AK-2	10.0±2.04 b	32.5±6.29 bc	67.2±10.33 a	100±0.0 a	62.5±4.78 b	88.8±3.75 a
AK-3	6.25±1.25 b	22.5±2.50 bc	80.0±7.90 a	95.0±2.04 a	55.0±6.45 b	81.3±7.73 a
AK-1b	6.25±1.25 b	15.0±2.88c	63.7±16.63 ab	86.2±10.48 a	52.5±4.78 b	76.3±8.98 a
AK-2b	10.0±2.04 b	25.0±5.0 bc	23.7±6.88 b	87.5±4.78 a	50.0±4.08 b	76.3±3.75 a
Ak-3b	6.25±1.25 b	15.0±1.25c	62.5±9.24 ab	85.0±5.00 a	62.5±4.78 b	82.5±4.33 a

Note: Values for the mean percentage (± SE) in a column, followed by the same letter, are not significantly different ($P \geq 0.05$) according to the complete randomized design procedures based on Tukey's test.
DAS= Days after sowing; WAT= Weeks after treatments

TABLE 2
The effects of CF and CRF on the percentage mortality of *Brassica rapa* seedlings at weeks 8, 9 and 10 after the treatments

Treatments	Percentage Kill **					
	8 WAT		9 WAT		10 WAT	
	10 DAS	14 DAS	10 DAS	14 DAS	10 DAS	14 DAS
CF	15.5±4.78a	90.0 ± 0.00 a	67.5±4.78ab	92.5±2.50 a	67.5±4.78 ab	92.50±2.5 a
AK-1	50.0±5.77 ab	75.0± 6.45 ab	75.0±2.88 a	97.5 ± 2.50a	75.0 ± 2.88 a	97.5 ± 2.30a
AK-2	27.5±6.29 ab	72.5± 2.59abc	60.0 ± 4.08ab	90.0± 4.08 a	60.0 ± 4.08ab	90.0 ± 4.08 a
AK-3	27.5±10.30ab	75.0 ± 2.88 ab	42.5 ± 8.53 b	87.5 ± 2.50a	42.5 ± 8.53 b	87.5 ± 10.4 a
AK-1b	17.5 ± 2.50 b	50.0 ±0.00 c	52.5± 7.50 ab	75.0 ± 10.4 a	52.5 ± 7.50 ab	75.0 ± 10.4 a
AK-2b	30.0± 9.12 ab	60.0 ± 10.8 bc	52.5 ± 4.78 ab	70.0 ± 5.77 a	52.5 ± 4.78 ab	70.0 ± 5.77 a
Ak-3b	40.0±8.53 ab	80.0 ± 4.08 ab	47.5 ± 7.50 ab	80.0 ± 9.12 a	47.5 ± 7.50 ab	80.0 ± 9.13 a

Note: Values for the mean percentage (± SE) in a column, followed by the same letter, are not significantly different ($P \geq 0.05$) according to the complete randomized design procedures based on Tukey's test.
DAS= Days after sowing; WAT= Weeks after treatments

all the treatments from evaluation at 9 and 10 WAT (see Table 2). All the treatments showed more than 70% mortality. Based on the evaluation at 10 DAS, higher mortality was observed at 9 WAT and 10 WAT as compared to the earlier treatments. This could be due to the release of diuron reaching the optimum and sufficient does was also available to give immediate effects on the mortality of the seedlings.

The immediate effect on seedlings' mortality declined at 16 WAT and 24 WAT (Table 3), indicating that time and cumulative effects of diuron from CRF played important roles in killing the seedlings. Meanwhile, the effect of CRF on the mortality of *Brassica rapa* seedlings was observed to be better than the CF treatments at 16 WAT. At 14 DAS, the 1mm granules with a ratio of 1:1 alginate and kaolin (AK-2) gave the highest mortality, i.e. 70%, as compared to the CF which gave 37.5% mortality. Only the AK-2b formulation gave the same level of mortality as that of the CF. This indicated that smaller granules release the diuron faster than the larger granules, giving it a better performance. This was primarily due to the larger surface area of the smaller granules. A similar result was also reported by Yousefzadeh *et al.* (1994) for the polyacrylamide hydrogel formulation, whereby smaller granules released active agent much faster than the bigger granules.

At 24 WAT (Table 3), the persistency of diuron was significantly prolonged in the soil when CRF was used. Only the CF showed significantly lower mortality at 14 DAS as compared to all the CRFs. Among the CRF, the mortality produced by AK-2 formulation (72.5%) was significantly higher than the AK-1 formulation (42.5%). This was clearly proven by the conventional diuron which had reduced its effectiveness with less than 50% of *B. rapa* being killed. However, all the CRF formulations gave better control than CF. The prolonged effectiveness of CRF is due to the diuron entrapped in alginate polymer which is protected against degradation agents such as sunlight, water, oxygen, and hydrolysis.

Similar results with CRF metribuzin by Kumar *et al.* (2010) showed that CRF stayed longer in soil than CF. The result indicated that the ratio of 4:0 alginate to kaolin in AK-1 released the active agent faster and presumably at greater quantity, depleting the diuron in the formulation. The present of kaolin could control the release of diuron because it could serve as a binder of the active ingredients (Gerstl *et al.,* 1994). The above results have shown that the 1mm granules of alginate and kaolin in a 1:1 ratio were the most suitable formulation and this combination was selected for further evaluation in subsequent experiments.

CONCLUSION

The release of diuron from alginate kaolin CRF was determined by a bioefficacy study which showed no significant influence of the size of granules. All the CRFs showed slower activity at the beginning of the experiment. This was no surprising as the CRF released their active agent over time, while in the CF treatment the active ingredient was immediately available upon application. At 15 and 24 WAT, AK-2 and AK-3 CRF showed higher percentages of the killed seedlings as compared to CF. Diuron in the CRF also persisted longer than the CF formulation. The increased persistence was attributed to the gradual release of the active ingredient, which at the same time, ensured minimal environmental loss due to leaching, volatilization and photo-degradation.

REFERENCES

Bahadir, M. (1987). Safe formulations of agrochemicals. *Chemosphere, 16*(2/3), 615-621.

Barret, P. R. F., & Logan, P. (1982). The localized control of submerged aquatic weeds in lakes with diquat-alginate. Sixth Europ. *Weeds Res. Soc. Int. Symp. On Aquatic Weeds,* 193-199.

Burril, L. C., Gardenas, J., & Locatelli, E. (1976). *Field manual for weed control research*. International Plant Protection Center, Oregon State University, Corvallis, Oregon, USA.

Connick, W. J. (1982). Controlled release of herbicides 2,4-D and Dichlobenil from alginate gels. *Journal Applied Polymer Science, 27*, 3341-3348.

Cotteril, J. V., Wilkins, R. M., & Dasilva, F. T. (1996). Controlled Release of Diuron from Granules Based on a Lignin Matrix System. *J. Controlled Rel.*, *5*, 23-36.

Dailey, J. O. D. (2004). Volatilization of alachlor from polymeric formulations. *Journal of Agricultural and Food Chemistry, 52*, 6742-6746.

Fernandez-Perez, M., Villa Franca-Sanchez, M., Gonzalez-Pradax, E., & Flores-Cespedes, E. (1999). Controlled release of diuron from alginate-bentonite formulation: water release kinetic and soil mobility study. *Journal of Agricultural and Food Chemistry, 40*, 791-798.

Garrido-Herrera, F. J., Gonzalez-Pradas, E., & Fernandez-Perez, M. (2006). Controlled Release of Isoproturon, Imidacloprid and Cyromazine from Alginate-Bentonite-Activated Carbon Formulations. *J. Agric. Food Chem.*, *54*, 10053-10053.

Hussain, M., Gan, J., & Rathor, M. N. (1992). Preparation of controlled release formulation of 14C-labelled thiobencarb herbicide and study of their environmental behavior. *Pesticide Science, 34*, 341-347.

Kumar, J., Nisar, K., Shakil, N. A., Walia, S., & Parsad, R. (2010). Controlled Release Formulations of Metribuzin: Release Kinetics in Water and Soil. *J. of Envir. Sc and Health Part B, 45*, 330-335.

Kulkarni, A. R., Soppimath, K. S., Aminabhavi, T. M., Dave, A. M., & Mehta, M. H. (2000). Glutaraldehyde crosslinked sodium alginate beads containing liquid pesticide for soil application. *Journal of Controlled Release, 63*, 97-105.

Kydonieus, A. (1980). Controlled release technologies: Methods, theory and applications. CRC Press.

Murphy, K. J., & P. R. F. Barrett. (1990). Controlled release formulations of aquatic herbicide. *Controlled Delivery of Drop Protection Agents*, 193-221.

Wan, L. S. C., Heng, P. W. S., & Chan, L. W. (1992). Drug encapsulation in alginate microspheres by emulsification, *Journal of Microencapsulation, 9*, 309.

Yousefzadeh, P., & Khadjari, M. S. (1994). Radiochemical preparation of polyacrylamide hydrogels and their use in agriculture. In Research and development of controlled release formulation of pesticide. IAEA, Vienna, I, 81-89.