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Association between Exposure to Pesticides and Cognitive Function in Greenhouse Workers (Case Study: Ahmadabad Village of Yazd Province)

Moslem Akhoundzardeini [1,2], Mohammad Javad Zare Sakhvidi [3,4], Fahimeh Teimouri [1], Mehdi Mokhtari [1*]

[1] Environmental Science and Technology Research Center, Department of Environmental Health Engineering, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[2] Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[3] Department of Occupational Health, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[4] Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) – UMR_S 1085, F-35000 Rennes, France.

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***Corresponding Author:**
Mehdi Mokhtari

Email:
mokhtari@ssu.ac.ir

Tel:
+983531492270

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ABSTRACT

Introduction: Exposure to pesticides in greenhouse workers is associated with several health outcomes, such as cognitive impairment. This study aimed to investigate the association between exposure to pesticides and cognitive function in Yazd city greenhouse workers and those living in the proximity of the greenhouses.

Materials and Methods: In this study, three groups of participants were selected, including the greenhouse workers, the residents in the proximity of the greenhouses, and the control group. A semi-quantitative assessment of pesticide exposure was used to calculate the subjects' cumulative pesticide exposure as a proxy for long-term exposure to pesticides. Blood level of acetylcholinesterase (AChE) activity was measured as a biomarker of effect. Frontal Assessment Battery (FAB), and Mini–mental State Examination (MMSE) were used to assess cognitive functions (including memory, executive functions, attention, visual, and verbal functions).

Results: Mean cholinesterase activity in the greenhouse workers (average = 7009.3 U/L) was lower than the two other groups. The results of the cognitive function score for both FAB and MMSE tests did not show a significant difference between the direct exposure and indirect exposure groups, although the cognitive function score in the pesticide applicators was lower than the two other study groups (e.g. for FAB score: 13.89, 14.55, and 15.4 for the greenhouse workers, the residents in the proximity of greenhouses, and the control group). The results also showed that in the direct exposure group, those with lower levels of cholinesterase activity also had a lower cognitive function.

Conclusion: The findings indicated that there is a potential link between impaired cognitive function and exposure to pesticides in the greenhouse workers.

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Introduction

The global volume of pesticide usage has increased significantly over the last decade. In addition to the increase in the number of pesticides used to combat pest resistance to pesticides, farmers use other methods, such as stronger concentrations of pesticides, increased frequency of pesticide usage, and increased combination of

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several pesticides [1, 2]. The World Health Organization (WHO) reported that in 2016, there were more than 150,000 deaths and 7,.000,000 poisoning cases caused by pesticide usage worldwide [3]. Ninety-nine percent of acute poisonings have occurred in developing countries, indicating the need to prevent exposure to pesticides. Farmers, agricultural sector workers, and their families are at higher risk of direct and indirect exposure to pesticides [4-6]. Greenhouse workers are also exposed to elevated levels of pesticides compared to other occupations, due to the use of pesticides in a relatively closed space, higher dosage of pesticide, and co-occurrence of exposure to pesticide with exposure to high temperature and humidity, which could increase the absorbance of these chemicals into the human body [7].

One of the most important health effects caused by exposure to pesticides is the permanent and irreversible damage to the nervous system [8-10]. The results of epidemiological studies have shown that there is a relationship between exposure to pesticides and the incidence of cognitive impairment [11, 12]. Neurological disorders are the most frequent (41%) health effects of pesticides exposure [13]. Organophosphates pesticides are one of the most widely used pesticides worldwide, are well-known for their effects on the central nervous system by inhibiting acetylcholinesterase (AChE), the enzyme that transfers acetylcholine levels [14-18]. Moreover, exposure to some organophosphates pesticides appears to be associated with chronic neurological complications [19].

There is some evidence that prolonged exposure to pesticides can cause serious neurological consequences, such as cognitive impairments, including verbal memory, nonverbal memory, prospective memory, psychomotor speed, selective attention, divided attention, and spatial functioning [20-22]. The results of the study by Dawson-Jaime Butler et al., showed that exposure to pesticides may be associated with learning disabilities, especially in motor functions[23]. In the study by Ebrahimzadeh et al., a significant difference was observed between the activity of cholinesterase enzyme in rice field workers and the control group [24].

The present study aimed to determine and compare the exposure to pesticides with cholinesterase activity and cognitive impairment, using semi-quantitative exposure assessment method. The subjects were divided into three groups, including those with direct exposure to pesticides (greenhouse workers), those with indirect exposure (residents in the proximity of the greenhouses), and those with no exposure (control group). Then, the association between pesticide exposure, AChE activity, and cognitive assessment results, including Frontal Assessment Battery (FAB) and Mini-mental State Examination (MMSE) was assessed.

Materials and Methods

Participants and study area

This cross-sectional study was carried out in 2019 in Ahmadabad village of Yazd province, Iran. The study population consisted of three groups with different pesticide exposure scenarios, including direct exposure group, indirect exposure group, and control group. The subjects in all the three groups were relatively similar in terms of age, gender, and health status. The sample size was calculated based on the statistical level of 0.05, and power of 80%, based on the effect size reported in the study by Sebastian et al.[16] They used the Rey–Osterrieth Complex Figure (ROCF) test for the assessment of cognitive function in a group of greenhouse workers [16].

Finally, 60 workers from the direct exposure group, 31 people of the indirect exposure group, and 30 people from the control group participated in the study. The inclusion criteria included having at least one year of work experience in a greenhouse for the direct exposure group, living at least one year in the proximity of greenhouses (at a distance of 3 to 8 km from the greenhouse) for the indirect exposure group, and having none of the above conditions in addition to not being employed in the agricultural sector for the control group [25]. Moreover, those with congenital, metabolic or chronic diseases, and family history of neurologic

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diseases were not included. We also excluded those who took any medication affecting the nervous system function. The participants were in the age range of 20-70 years.

Exposure assessment

Direct exposure assessment was not done in this study due to the difficulty of accessing analytical methods for measuring pesticides. Therefore, the modified version of a semi-quantitative pesticide exposure model was used based on the study conducted by Negatu et al. in Ethiopia [26]. A semi-quantitative pesticide exposure algorithm has been extensively used and evaluated by different field monitoring studies and has had a good correlation with post-application urinary concentration of pesticide biomarkers. This is an inexpensive and easily adaptable method for pesticide applicators and farm workers exposure assessment. Cumulative applicator exposure was defined by multiplying variables of greenhouse workers' work experience, the area under cultivation per year, and spraying frequency in a recent month (Equation 1).

Cumulative applicator exposure = work experience× area under cultivation× spraying frequency (Equation 1)

Outcomes assessment

AChE activity: AChE activity related to pesticide exposure in the three groups were assessed base on the German Society for Clinical Chemistry (DGKC) standard method [27]. Venous blood (5 ml) was taken from all the participants. Blood samples were centrifuged at 3000 rpm for 10 minutes and serum was separated. The cholinesterase assay was performed on a diluted sample using butyrylthiocholine substrate present in the reagent to form butyrate and thiocholine. Thiocholine, in presence of potassium hexacyanoferrate (III), reduces the yellow-colored hexacyanoferrate III to colorless potassium hexacyanoferrate (II). The decrease in absorbance is directly related to the activity of serum cholinesterase in the sample (All the procedures was done in a certified medical laboratory).

Cognitive outcomes

The FAB and MMSE were used to assess the cognitive function of all three study groups. The FAB test included 6 questions related to similarities (conceptualization), lexical fluency (mental flexibility), motor series "Luria" test (programming), conflicting instructions (sensitivity to interference), go/no-go (inhibitory control), and comprehension behavior (environmental autonomy). Each FAB test score was between a minimum of 0 and a maximum of 3 and the total score was of maximum of 18 [28]. The MMSE test is a 20-point questionnaire used to evaluate different cognitive domains, such as memory, visual function, executive function, and *etc*. Each question of the MMSE test was scored as either correct or incorrect; the total score ranged from 0 to 30, the higher score represents better cognitive performance [29].

Covariates

The data were collected by a researcher-made checklist comprised of six sections, including demographic information of the participants, the way of preparing pesticides, the way of working with pesticides, using personal protective equipment's during spraying, individual health, and specifications of the greenhouse where the participants work. Moreover, information of indirect exposure and control groups was obtained by another questionnaire, including demographic characteristics, disease history, and tobacco and alcohol use.

Data analysis

The data were analyzed by R software version 3.5, using one-way multivariate test (ANOVA), t-test, and Tukey post hoc test. Multiple linear regression models were used to determine the relationship between exposure, and AChE, MMSE, and FAB as outcome. The significance level was considered 0.05 in all tests. Three different regression models were defined according to the level of adjustment. The first model was the crude model. The second model (model 1) was adjusted for age and education. The third model (model 2) was additionally adjusted for smoking status and body mass index (BMI).

Ethical Issue

Informed consent was taken from all the participants before participating in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of School of Public Health, Shahid Sadoughi University of Medical Sciences (IR.SSU.SPH.REC.1397.053 - 2018-08-04).

Results

The demographic information of the participants is presented in table 1. All the subjects were male and their age was in the range of 32-35 years (mean = 33.24). Most of the participants (89 %) had high school and lower education. The highest BMI was for the indirect exposure group, and the highest prevalence of smoking (18%) was for the direct exposure group.

We found better cognitive performance in those with higher education (Figure 1).

Table 1: Demographic information of the subjects

Variable	Direct exposure group (N = 60)	Indirect exposure group (N = 31)	Control group (N = 30)	Total (N = 121)	P-value
Age	32.05 (7.14)	32.84 (10.21)	34.83 (7.43)	32.94 (8.11)	0.360
Education					< 0.001
Illiterate	9 (15.0%)	0 (0.0%)	0 (0.0%)	9 (7.4%)	
Primary school	16 (26.7%)	8 (25.8%)	0 (0.0%)	24 (19.8%)	
Junior high school	16 (26.7%)	11 (35.5%)	8 (26.7%)	35 (28.9%)	
High school	14 (23.3%)	8 (25.8%)	18 (60.0%)	40 (33.1%)	
University	5 (8.3%)	4 (12.9%)	4 (13.3%)	13 (10.7%)	
Smoking					0.502
Non-smoker	51 (85.0%)	28 (90.3%)	29 (96.7%)	108 (89.3%)	
Smoker	8 (13.3%)	3 (9.7%)	1 (3.3%)	12 (9.9%)	
Ex-smoker	1 (1.7%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	
BMI (kg/m^2) *	24.67 (3.46)	27.16 (4.33)	25.83 (2.87)	25.60 (3.69)	0.039

Note: For the continuous variable the numbers are mean (standard deviation) and for the rest Variable the numbers are frequency (percent).
* Body Mass Index

AChE activity*:* The mean AChE activity in the three groups (direct exposure, indirect exposure, and control) is shown in Table 2. The mean AChE activity was lower in the direct exposure group compared to the indirect exposure and control groups. The AChE activity was approximately equal in the indirect exposure and control groups. There was no statistically significant difference between the cholinesterase activity in the three groups (p-value = 0.20).

Cognitive function

The results of cognitive function assessment (FAB and MMSE tests) of the three groups are presented in Table 2. The results showed a statistically significant difference between the three groups according to the MMSE test findings (p-value < 0.001). Moreover, lower FAB test score was found in the direct exposure group compared to the other groups; however, the difference was not significant. According to the FAB test, there was no difference in cognitive function between the direct exposure and indirect exposure groups; however, there was a difference in the direct exposure and control groups. The FAB test mean score in the proximity group was 0.56 higher than in the direct exposure group, although this difference was not significant. Additionally, compared to the control group, the mean FAB score was 1.05 higher than the direct exposure group, which was statistically significant. According to the MMSE test, there was no significant difference between the direct exposure and indirect exposure groups and their mean difference was 1.01. The MMSE test score in the control group was 2.55 score higher than the direct exposure group and there was a significant difference between the two groups. Moreover, the control group score was 1.54 higher than the indirect exposure group which was statistically significant.

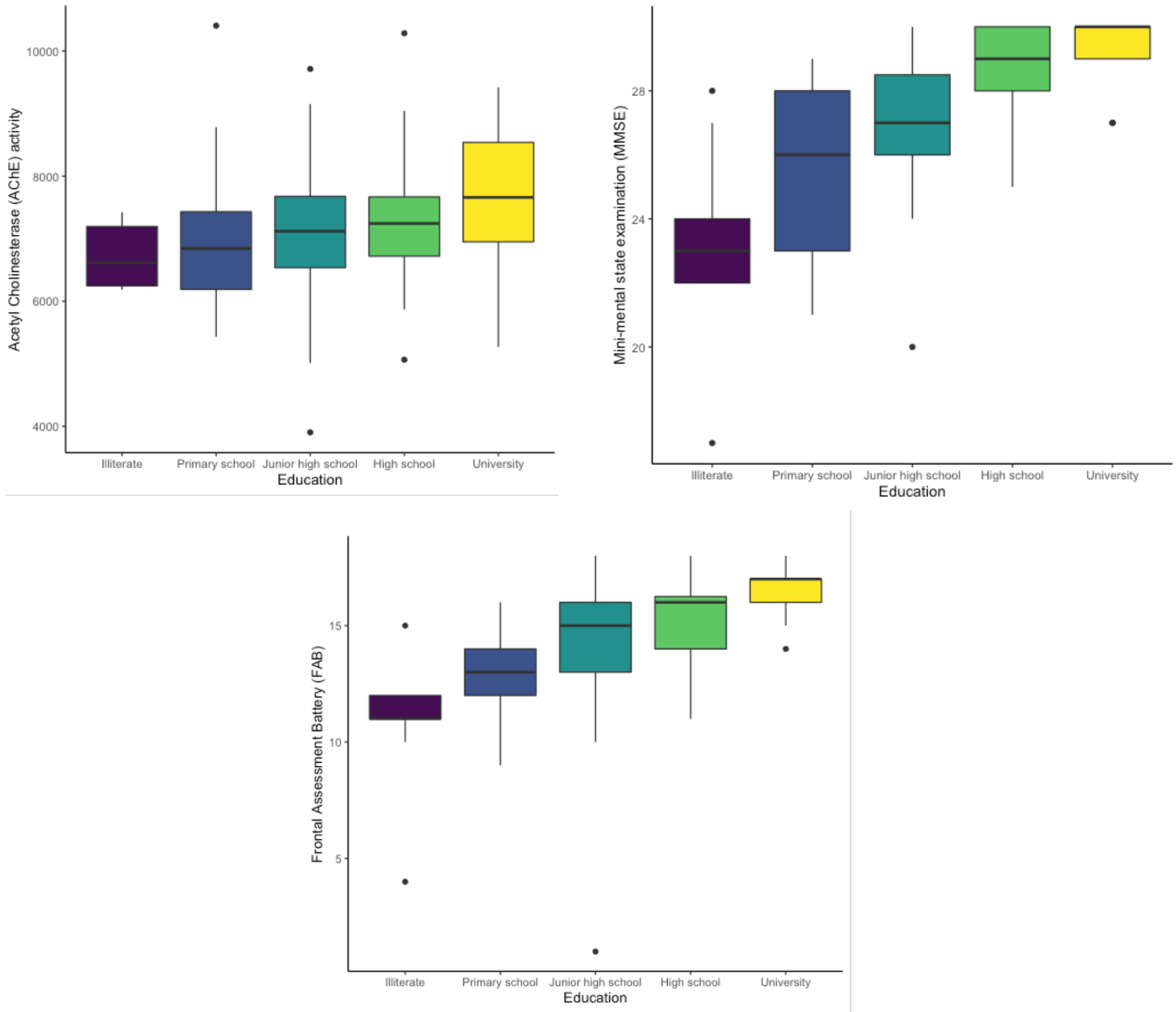

Figure 1: Acetylcholinesterase activity (U/L) and Mini–mental State Examination and Frontal Assessment Battery scores according to different levels of education

Table 2: Mean Acetylcholinesterase activity, and cognitive function (based on Frontal Assessment Battery and Mini–mental State Examination tests) of the three groups

Variable	Direct exposure group (N = 60)	Indirect exposure group (N = 31)	Control group (N = 30)	Total (N = 121)	P-value
Cholinesterase (U/L)	7009.30 (997.58)	7282.21 (1325.25)	7254.05 (895.96)	7139.90 (1067.41)	0.199
MMSE*	26.28 (2.78)	27.29 (2.15)	28.83 (1.49)	27.17 (2.56)	< 0.001
FAB**	13.88 (3.10)	14.55 (2.29)	15.43 (1.55)	14.44 (2.65)	0.057

Note: For the continuous variable the numbers are mean (standard deviation) and for the rest Variable the numbers are frequency (percent).

* Mini–mental State Examination

** Frontal Assessment Battery

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Regression analyses

The association between cognitive function score and exposure rate was also examined in the direct exposure group and its results are presented in table 3. Exposure values were calculated based on the Equation 1. According to the results, there was no significant association between cognitive function score (both FAB and MMSE) and greenhouse exposure. However, the results for models adjusted for other covariates (model 1 and model 2) for FAB were significant, and showed negative association between cumulative pesticide exposure and cognitive function.

Table 3: The association between cognitive function and cumulative pesticide exposure index

Model	MMSE*	FAB**
Crude model	0.232 (-0.440: 0.904)	0.027 (-0.725: 0.778)
Model 1	0.023 (-0.525: 0.570)	-0.153 (-0.851: 0.546)
Model 2	0.015 (-0.557: 0.587)	-0.218 (-0.944: 0.508)

Note: Model 1is adjusted for age and education. Model 2 additionally adjusted for smoking and BMI. All the effect sizes are reported based on an interquartile range increase in exposure index (1495600).

* Mini–Mental State Examination

** Frontal Assessment Battery

Discussion

In this study, we found that the mean AChE activity in the direct exposure group was lower than the other two groups and the mean was approximately equal in the indirect exposure and control groups. The lower activity of cholinesterase enzyme in greenhouse workers can be an indication of their exposure to pesticides, leading to the production of free radicals and oxidative stress and confirms the absorption of pesticides in their bodies [30]. Other similar studies have been conducted in high-risk regions. The results of these studies have shown that the acetylcholinesterase activity of farmers is probably lower than that of the general population [31, 32].

The results of cognitive assessment in this study showed that the cognitive function score for MMSE was significantly different in the direct exposure and control groups and the mean FAB score in the control group was 1.55 point higher than the direct exposure group. The mean MMSE score in the control group was 2.55 point higher than the direct exposure group. The results of investigating the relationship between pesticide exposure and neurological cognitive function in greenhouse workers showed a decrease in processing speed, poorer function in visual and motor coordination, and visual and working memory skills compared to the control group. Therefore, exposure to pesticides potentially has adverse health effects on individuals' mental and behavioral functions. Similar studies have been conducted on farmers' cognitive function, which are consistent with the results of the present study. Ismail et al., reported that the results of all functional tests, such as attention, visual, verbal, and perceptual showed a significant decrease in neurobehavioral function in the exposure group compared to the control group [33].

The results of a study on Chinese farmers showed that poisoning with pesticides is associated with reduced response rates, short-term memory deficits, as well as loss of coordination. Poorer function in hearing, speed, accuracy, and visual perception tests was observed in the direct exposure group compared to the control group [34]. MMSE cognitive test results in the study of Baldi et al. showed that chronic exposure to pesticides reduces cognitive function, and long-term exposure causes the risk of dementia [35], which is in line with the results of the present study.

Limited studies have been conducted on the health effects of pesticides on the residents in the proximity of agricultural areas. The results of the cognitive assessment showed that the cognitive function score for both the FAB and MMSE tests in the indirect exposure group was higher than the direct exposure group; however, this difference

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was not significant. Furthermore, the FAB cognitive test score was not significantly different in the indirect exposure and control groups; however, the MMSE test showed a significant difference between the two groups. This difference might be due to the fact that the MMSE test covers a broader set of cognitive domains [36]. Sebastian et al. reported that the direct exposure group performed significantly poorer in the executive function, verbal fluency, and visual and auditory memory tests compared to the indirect exposure group. The indirect exposure group was also at a lower level than the control group. Therefore, both direct and indirect exposure groups are affected by exposure to pesticides [16].

One of the important factors indicating the vulnerability of greenhouse workers is the number of years of formal education. Both FAB and MMSE cognitive tests were significantly correlated with education level. The results showed that the level of cholinesterase activity in the indirect exposure and control groups was significantly higher in the subjects with higher education level. Even in the indirect exposure group, the relationship was almost linear; since people with a higher education level were more aware or afraid of the health effects of pesticide exposure; therefore, they were less exposed to pesticides. The results of Sharifi et al.'s study showed that one of the factors affecting the use of pesticides is the level of education and awareness [37].

In the direct exposure group, there was no relationship between education level and the cholinesterase enzyme activity. However, using pesticides affects the health of low-educated people due to their inability to understand the guidelines, lack of awareness of how to read, or lack of information about the health effects of pesticides on the human body and the environment [38]. However, the results of this study showed that academic education is not a good proxy for people's health literacy level. In an interview with greenhouse workers, it was found that they did not use appropriate personal protective equipment, such as masks, long-sleeved gloves, long boots, hats, face covers, and regular shower after spraying and their only safety tool was some kind of cloth mask. Therefore, their exposure to pesticides increases and their cholinesterase activity decreases. In a similar study, Vikkey et al. reported that education level did not affect the level of cholinesterase activity in cotton field workers [39].

The FAB and MMSE cognitive test scores were significantly correlated with the education level, and those with higher education levels scored higher on each test. People with higher education usually have better understanding and expression in cognitive testing. This may lead to better function in cognitive tests and consequently later detection of pesticide effects on cognition [40]. In the study of Cheng et al., higher education level was associated with better scores on MTA (medial temporal lobe atrophy) cognitive test [41]. In Carolyn et al.'s study, the MMSE cognitive test score was lower for people with lower education level [42]. The results of these studies were consistent with the current study. In the direct exposure group, people with lower cholinesterase activity levels also had poorer cognitive function. However, the obtained results were not significant, which might be due to the limitations of the study and the lack of generalization of small sample size to the whole population. However, most of the previous studies have reported that occupational exposure to pesticides is associated with cognitive impairment[43-47]. The results of the study by Kim et al. showed that exposure to chlorine organic pesticides was associated with the cognitive function measured by the Digit Symbol Substitution Test (DSST) in the US elders; however, it was not significant [48]. The results of DSST and FAB tests were consistent with our study.

Factors that have neurotoxic effects can cause a variety of problems, including mental retardation and disability, as well as changes in cognitive function, which largely depend on exposure to the chemical [49]. According to the results, there was no significant relationship between FAB cognitive function score and MMSE of direct exposed subjects and exposure rate. Many other studies have shown a negative relationship between exposure to pesticides and various neurobehavioral functions [43, 50, 51], which is not in line with the findings of the present study, due to the intrinsic characteristic of the individuals [33]. In the study by Ismail et al., no significant relationship was found between individuals' exposure and neurological function tests [52], which is consistent with the results of the current study.

Study limitations

This study had several limitations. First the sample size in the study was small and part of the null findings could be due to the power of the study. Second, although it was tried to use a previously used semi-quantitative exposure assessment method in the study, the validity of the approach was not tested in the target population and the obtained results by this method could be subject to exposure misclassification. Previous studies have shown that exposure to different pesticides is associated with cognitive impairment in different domains. However, in this study a general MMSE test was used which provides a clue about general cognitive impairment rather than domain-specific outcomes. However, the results of the more specific test (such as FAB) should also be interpreted with caution due to the limitation in the sample size.

Conclusion

This study aimed to determine the association between pesticide exposure and AChE activity and cognitive function in greenhouse workers and those living in the proximity of the greenhouses. The findings on the reduced level of cholinesterase activity in the greenhouse workers have been highlighted in previous studies. The findings on poorer cognitive function in the greenhouse workers and more importantly the residents in the proximity of the greenhouses are of great importance, which should be explored in future studies with larger sample size and using better exposure and outcome assessment methods. These findings highlight the importance of safety and health improvement programs for pesticides applications in agricultural sectors, especially greenhouse activities.

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Conflict of interest

The authors declare that they have no conflict of interest.

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