Please note that the following annotated bibliography is presented sequentially with secondary literature being first, and the remaining primary literature in groups based on the kind of insecticide studied in each article. Order of insecticide presented in this bibliography are phenylpyrazole varieties, neonicotinoid varieties, and an organophosphorus variety.

Reference:

Fikadu, Z. (2020). Pesticide use, practice and its effect on honey bee in Ethiopia: A review. *International Journal of Tropical Insect Science, 40*, 473-481. https://doi.org/10.1007/s42690-020-00114-x

Summary (300 words):

This secondary article reviews research surrounding the impacts of a variety of pesticides on honeybees in Ethiopia, as well as the greater economic and societal impacts of pesticide use in general. Pesticides, particularly insecticides, are a common, and generally effective form of deterrent against insects that could reduce the yield and productive efficiency of crops. Typically, pesticides are deployed aerially to maximize their effective range, and oftentimes amongst smaller farm operators, different types of pesticides are mixed together to save time and labour. Incorrect practices, such as pesticide mixing, application up to four times a year, or simply excessive usage, however, has led to marked negative impacts on environmental welfare, especially in the realm of pollinators.

The results of improper pesticide application are definitive, with as many as three-quarters of beekeepers losing their honeybee colonies since the introduction of pesticide use in Ethiopia as a result of detrimental behavioural changes in honeybees. Furthermore, honeybees are more heavily impacted by pesticides than the chemicals' target organisms, thus, farmers may lose track of the underlying cause of poor crop yields. Ironically, pesticide use is inadvertently limiting the yield of crops as honeybees are vital to agricultural productivity. Pesticide use may reduce crop losses by target organisms, but in doing so, future productivity is hindered as a result of the disproportionate impact of these pesticides on important pollinators, such as honeybees. Three methods could mitigate the harmful effects of pesticides, and are as follows; insecticides of relatively low toxicity could be substituted for existing ones, applying insecticides toxic to honeybees during plant blooming could cease, and legislatively approved application methods could be more widely used. These methods, alongside better law enforcement and improved training amongst farmers, can help to curb the dangerous possibility of a world with too few pollinators.

Contribution (100 words):

This article summarizes the effects of pesticide use on honey bees and outlines broadly the behavioural, cognitive, and physiological changes that can be expected in honeybees exposed to insecticides. How pesticides impact honeybee colonies, honeybee products such as honey or wax, and how pesticides contribute to colony collapse disorder was also discussed. Crucially, the article also outlined three strategies for mitigating the impact of pesticides on honeybees. Additional methods to mitigate pesticide impact could be explored, however. This article also serves as a fantastic source of primary literature on the topic of honeybees affected by pesticides.

Reference:

Poquet, Y., Vidau, C., & Alaux, C. (2016). Modulation of pesticide response in honey bees. *Adipologie, 47*, 412-426. https://doi.org/10.1007/s13592-016-0429-7

Summary (300 words):

This secondary article reviews research surrounding the impacts of pesticides on honey bees when exposed bees are also afflicted with additional stressors. Pesticides, while efficient in protecting plants and minimizing crop yield losses, can often have detrimental effects on crop growth due to their effect on pollinators, such as honey bees. The specific impacts of concurrent exposure of honey bees to pesticides and other adverse factors are poorly researched, and understanding how pesticides increase bees' vulnerability to natural aggravation and vice versa is necessary. This study conducted a meta-analysis, examining previous research on how bees are affected by pesticides, and especially how pesticide effects might change as a result of exposure to other challenges, such as parasites or extreme weather.

Numerous trends were identified and pesticides were clearly implicated in worsening the deleterious effects of pre-existing challenges to the survival of bees. The review indicates that pesticides could worsen the effects of cold weather, for instance, and correspondingly, cold weather can worsen the effects of pesticides. Furthermore, the review showed that pesticides may compromise bees' ability to fight off pathogens, making parasitic infections more common when foragers return to the hive with diseases. Of note was the fact that bees delegated to foraging duties are, in particular, more susceptible to infection as a result of increased pesticide exposure. In turn, increased forager attrition might result in reduced food stores and a greater likelihood that hives will perish while trying to adapt to fewer resources that are immediately available. The harmful impacts of pesticides when combined with secondary stressors are extensive, and the review recommends future research into understanding how high bee mortality might result upon exposure to pesticides below a lethal dosage due to the aforementioned coaction.

Contribution (100 words):

This article summarizes how pesticides impact, and usually worsen, the challenges of environmental and endogenous co-stressors that honey bees face. Nonetheless, it is also noted that with an improved understanding of how pesticides and co-stressors interact, more consideration can be made to how the toxicity of pesticides might be modified to reduce effects on honey bees. This study concurs with others on this subject that pesticides have negative effects on honey bees, especially when paired with other co-stressors. This article was included in this review because of its discussion of pesticides and their cooperative effects on honey bee co-stressors.

Reference:

El Hassani, A.K., Dacher, M., Gauthier, M., & Armengaud, C. (2005). Effects of sublethal doses of fipronil on the behaviour of the honey bee (*Apis mellifera*). *Pharmacology Biochemistry and Behavior, 82*(1), 30-39. https://doi.org/10.1016/j.pbb.2005.07.008

Summary (300 words):

This primary article investigated the effects of fipronil, an insecticide of the phenylpyrazole class, on honey bee behaviour. Numerous pre-existing studies have established that insecticides such as fipronil, a phenylpyrazole insecticide, can increase bee mortality directly. However, additional study into the effects of doses below lethal levels is warranted to understand how insecticides might increase mortality indirectly. El Hassani et al. (2005) postulated that indirect effects of fipronil lead to behavioural changes that, in turn, reduce honey bee survivability. It was the goal of the study to elucidate what kind of behaviour fipronil affects, and to what extent those behaviours change. Assessment of locomotor activity was accomplished using an open field-like apparatus, with bee movements being recorded every five seconds. Sucrose sensitivity was analyzed by measuring the tendency of the bees' to extend their proboscis in response to sucrose, and, lastly, olfactory learning and memory were assessed using Pavlovian conditioning of the bees' proboscis extension reflex. A coffee odour represented the conditioned stimulus in this case. Honey bees were exposed to the insecticide either by topical application to the thorax, or orally in doses of no more than 1ng/bee.

Fipronil was found to significantly reduce proboscis extension temporarily when administered in small doses. Fipronil was also found to play a notable role in olfactory learning and memory when topically applied, with moderate doses of the insecticide causing statistically significant impairments to memory formation. This study is noteworthy because it outlined the effects of fipronil in varying doses and modalities of exposure on three honey bee behaviours crucial to the species' survival. The researchers did recommend two kinds of experiments moving forward, namely, an analysis of chronic fipronil treatment under laboratory conditions, and field experiments.

Contribution (100 words):

This article provides new insight into the effects of the fipronil insecticide in particular, and how this substance can influence bees' sucrose sensitivity and olfactory memory formation under conditions of moderate topical exposure. Furthermore, this study reinforced the pre-existing notion that the impacts insecticides have on honey bee dynamics are complex and dose-dependent. Lastly, the results of this study provide a comprehensive framework that future studies can employ when analyzing the effects of chronic fipronil exposure in controlled laboratory conditions, as well as chronic fipronil exposure in the field.

Reference:

Aliouane, Y., El Hassani, A.K., Gary, V., Armengaud, C., Lambin, M., & Gauthier, M. (2009). Subchronic exposure of honeybees to sublethal doses of pesticides: Effects on behaviour. *Environmental Toxicology and Chemistry, 28*(1), 113-122. https://doi.org/10.1897/08-110.1

Summary (300 words):

This primary article investigated the effects on honey bee behaviour of sublethal doses of three different insecticides; fipronil, acetamiprid, and thiamethoxam. Specifically, Aliouane et al. (2009) sought to uncover how oral and topical (by application to the thorax) exposure to these insecticides influenced the locomotor activity, water consumption and responsiveness, sucrose responsiveness, olfactory learning, and mortality of honey bees. Quantities of insecticide ranged from one-fifth of the median lethal dose to one-five-hundredth. The research team explored how the above behaviours were influenced using parallel assays in laboratory-controlled settings– both the control group and the group exposed to insecticide were observed for their locomotion using specially adapted software. Water and sugar responsiveness, water consumption, and olfactory learning were tested using the bees' proboscis extension reflex as an indicator of cognitive function.

Fipronil was found to be the most impactful insecticide tested with one-five-hundredth of the lethal dose administered topically causing a significant increase in immobility and water consumption. Fipronil also contributed to increased olfactory generalization, reducing olfactory learning. Thiamethoxam induced short-term memory loss when in one-five-hundredth median lethal dosage, or impairment to learning performance in one-fifth dosage administered topically. One-fifth dosage of thiamethoxam administered orally also contributed to reduced sucrose responsiveness. Acetamiprid, when administered orally, was found to cause increased responsiveness to water. This study was significant in that it outlined the impact of exposure to three commonly used insecticides on honey bee behaviour that is crucial to colony health, and demonstrated the risks of exposing honey bees to fipronil, especially. This study also noted how the effects of exposure change with increasing quantities of each insecticide. Future studies could focus on how the harmful effects of honey bee exposure to these insecticides might be mitigated.

Contribution (100 words):

This article provides new insight into the different effects of fipronil, thiamethoxam, and acetamiprid insecticides on honey bee behaviour crucial to bee colony function and overall health. Furthermore, the differences in the effects of each insecticide at one-fifth of the lethal median dosage, and one-five-hundredth the lethal median dosage were articulated, allowing for identification of the no-observed-effect-concentration for behavioural assays in the case of acetamiprid and thiamethoxam at 3 mg/L and 3 μg/L, respectively.

Reference:

El Hassani, A.K., Dacher, M., Gary, V., Lambin, M., Gauthier, M., & Armengaud, C. (2008). Effects of Sublethal doses of acetamiprid and thiamethoxam on the behaviour of the honeybee (*Apis mellifera*). *Archives of Environmental Contamination Toxicology, 54*(4), 653-661. https://doi.org/10.1007/s00244-007-9071-8

Summary (300 words):

This primary article investigated the effects of two neonicotinoid insecticides, namely, acetamiprid and thiamethoxam, on honey bee behaviour. Pre-existing studies have established that neonicotinoids can increase bee mortality directly. However, studying the effects of doses below lethal levels is warranted to understand how these insecticides might increase mortality indirectly. El Hassani et al. (2008) postulated that indirect effects of acetamiprid and thiamethoxam lead to behavioural changes that reduce honey bee survivability. The study's objective was to determine what kind of behaviour these insecticides effect, and to what extent those behaviours change. Assessing locomotor activity was accomplished using an open field-like apparatus, with bee movements being recorded every five seconds. Sucrose and water sensitivity were analyzed by measuring the bees' tendency to extend their proboscis in response to sucrose or water, and, lastly, olfactory learning and memory were assessed using Pavlovian conditioning of the bees' proboscis extension reflex. A coffee odour represented the conditioned stimulus. Honey bees were exposed to the insecticide either by topical application to the thorax, or orally in doses of 0.1-1μg/bee of acetamiprid, or 0.1-1ng/bee of thiamethoxam.

Topical application of acetamiprid significantly increased the distance walked in the open field-like apparatus, and for both insecticides, topical application at any dose resulted in a significant decrease in mobility compared to oral application. Oral application of acetamiprid decreased proboscis extension in response to sucrose and increased response to water. Interestingly, continued moderate topical exposure of thiamethoxam correlated with increased olfactory learning and memory performance. This study notably reinforces the dose-dependent relationship of neonicotinoid insecticides seen in other behaviours. This study also highlighted that acetamiprid impacts honey bee behaviour more dramatically than thiamethoxam. The researchers recommend additional study into understanding why acetamiprid has more deleterious effects than thiamethoxam.

Contribution (100 words):

This article was successful in demonstrating the influence that acetamiprid and thiamethoxam insecticides have on locomotion, sucrose and water responsiveness, and olfactory learning and memory. Furthermore, this study has indicated that acetamiprid has a much more pronounced effect on the aforementioned behaviours than does thiamethoxam. The effects of acetamiprid and thiamethoxam as neonicotinoid insecticides are consistent with other findings. This article was included in this review because it discusses the influence of neonicotinoid insecticides on honeybee behaviour that is crucial to the survival of the species, and the ability of honey bees to perform their ecological function as pollinators.

Reference:

Lambin, M., Armengaud, C., Raymond, S., & Gauthier, M. (2001). Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honey bee. *Archives of Insect Biochemistry and Physiology, 48*(3), 129-134. https://doi.org/10.1002/arch.1065

Summary (300 words):

This primary article examined the effects of imidacloprid, an insecticide of the neonicotinoid class, on the honey bee at low doses. Several studies have established the lethal effects of imidacloprid on honey bees, as well as how this insecticide can affect visible behaviour such as detection of sucrose via sensation, and movement via motor structures. The impact of imidacloprid on learning, however, especially at low doses, has been poorly researched. Lambin et al. (2001) were interested in understanding how imidacloprid can influence underlying learning behaviours, such as habituation and sensitization. A display of the proboscis extension reflex in response to sucrose was used as an indicator for such learning. The lowest concentration of sucrose that elicited proboscis extension was determined to be the threshold for comparison, and is a limit to taste sensitivity. This study also considered the bees' motor activity using an open field-like apparatus, within which the bees' movements were recorded every five seconds. Doses of insecticide ranging from 1.25ng/bee to 20ng/bee were used for both behavioural assessments.

Higher doses of imidacloprid, that is in excess of 5ng/bee, were found to cause a significant increase in the bees' taste sensitivity. Furthermore, doses of insecticide less than 2.5ng/bee were shown to increase mobility, however, doses over this amount were shown to decrease mobility. This study is significant in that it emphasises imidacloprid's dose-dependent effect on honey bee behaviour in terms of movement and taste sensitivity, with low doses resulting in negligible effects, but higher doses past a threshold of roughly 2.5ng/bee cause impairments to both behaviours. The researchers recommend that additional experiments be conducted to assess the more general effects of imidacloprid on behaviours other than movement and taste sensitivity.

Contribution (100 words):

This article provides new information on the effects of imidacloprid on non-associative learning abilities of the honey bee, and describes the dose dependent effect this insecticide has on learning and mobility. The negative effect of imidacloprid on honey bee learning and mobility seen in other articles on this subject are supported by the results of this study. This article in particular was included in this review because of its description of the effects of non-lethal dose of insecticides, particularly a neonicotinoid, which can be cross-referenced with the results of other studies to establish the wide-ranging effects of this insecticide.

Reference:

Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, & Pham-Delegue, M. (2004). Imidacloprid impairs memory and brain metabolism in the honey bee (*Apis mellifera* L.). *Pesticide Biochemistry and Physiology, 78*(2), 83-92. https://doi.org/10.1016/j.pestbp.2003.10.001

Summary (300 words):

This primary article investigates the effects of imidacloprid, an insecticide of the neonicotinoid class, on the honey bee at multiple doses. Various studies have noted the lethal effects of imidacloprid on honey bees, as well as how this insecticide can impair learning in a variety of circumstances. Given the range of differential effects imidacloprid can have, the dynamic between honey bees and this insecticide is deserving of additional research. Decourtye et al. (2004) are, thus, interested in detailing more specifically how imidacloprid influences the memory of honey bees. Imidacloprid at doses of either 0.12ng/bee or 12ng/bee were administered to bees at the beginning of the first two experimental trials, and part way through a third. The first experiment assessed bees' ability to recall their exposure to sucrose after a brief delay. The second experiment examined the bees' ability to recall the same stimulus over a day, while being tested for retention at multiple intervals. The third experiment also assessed the same stimulus over a day, however exposure to the insecticide occurred temporally near the retention test rather than at the beginning of the experiment.

Imidacloprid was found to impair medium-term memory formation at higher doses when compared to the lower dose and control. This was elucidated based on the reduced performance of bees subjected to higher doses of the insecticide. A hypothesis that memory formation was impacted, rather than retention, stemmed from the fact that neither the short term or long term memories of bees were affected, regardless of treatment. Thus, imidacloprid was suggested to impair the bees' conversion of short-term memories to medium-term memories. This study was significant in that it articulated the effects that imidacloprid has on olfactory memory. Future research is recommended to understand the mechanism of how imidacloprid disrupts olfactory memory formation.

Contribution (100 words):

This article demonstrated the advantages of using staining of the cytochrome oxidase enzyme in brain regions associated with olfactory learning and memory when determining the effects of an insecticide on olfactory learning and memory. Furthermore, this article submits a hypothesis to explain why imidacloprid only affects medium-term memory rather than short or long-term. Though other studies have scarcely used the cytochrome oxidase enzyme to study insecticide effects, the general impact of imidacloprid is consistent with other findings. This article is included in this review because of its use of enzyme staining to study insecticide effects on honey bee behaviour.

Reference:

Schnider, C.W., Tautz, J., Grunewald, B., & Fuchs, S. (2012). RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behaviour of *Apis mellifera*. *PLoS ONE, 7*(1), e30023. https://doi.org/10.1371/journal.pone.0030023

Summary (300 words):

This primary article examines how imidacloprid and clothianidin neonicotinoid insecticides affect the foraging behaviour of honey bees. Numerous studies have corroborated the impact of neonicotinoids on honey bee mortality, however, further research is required in understanding how these insecticides influence behaviour, and how these behavioural changes might lead to reduced survivability of bees. Schnider et al. (2012) were interested in understanding how imidacloprid and clothianidin affect the foraging behaviour of honey bees in particular, using three different metrics. Doses of insecticides used ranged from 0.15ng/bee to 6ng/bee for imidacloprid, and 0.05ng/bee to 2ng/bee for clothianidin, and were administered to bees orally. The experimental treatment involved observing bees placed in an open-field-apparatus which included a beehive distanced seven meters away from a sucrose solution dispensing feeder. Radio frequency identification transponders, also known as RFID transponders labeled each bee. These transponders provided comprehensive monitoring of individual bees regardless of treatment and were able to track how many bees were returning to their hive. Other important movements tracked were the number of times bees visited the feeder, and how long bees spent foraging.

Both imidacloprid and clothianidin significantly reduced hive visits and feeder visits in all but the smallest dose, and extended the time between foraging trips indicating a reduction in foraging efficiency.This study is significant because it outlines the process of using RFID transponders to study bees' individual foraging habits in laboratory settings, and provides an introduction to using this method of study in the field. Furthermore, this study demonstrated that the effects of clothianidin are more potent than those of imidacloprid. The researchers recommend additional studies to take advantage of this novel RFID transponder technology to examine how insecticide effects might be more prominent when food sources are further away from hives.

Contribution (100 words):

This article outlines the impacts that imidacloprid and clothianidin neonicotinoid insecticides have on three behaviours crucial to honey bee foraging; the proportion of bees returning to their hive, the number of visits to a feeder bees make, and the time between foraging trips. Furthermore, this study provides a framework for employing RFID transponder technology when monitoring foraging behaviour of individual bees. This article generally supports the findings of other studies on the effects of neonicotinoid insecticides on honey bee foraging. This article was included in this review due to its novel use of RFID tracking to study foraging behaviours.

Reference:

Decourtye, A., Lacassie, E., & Pham-Delegue, M. (2003). Learning performances of honey bees (*Apis mellifera L*) are differentially affected by imidacloprid according to the season. *Pest Management Science, 59*(3), 269-278. https://doi.org/10.1002/ps.631

Summary (300 words):

This primary article examined how the neonicotinoid insecticide imidacloprid affects olfactory learning performance in two different varieties of honey bees. Previous research has outlined the impact of imidacloprid on honey bee olfactory memory, however, further study is needed to understand how this insecticide, and one of its metabolites, might induce distinct behavioural changes in different honey bee varieties. Consequently, Decourtye et al. (2003) investigated how winter and summer honey bees are influenced by imidacloprid and 5-OH-imidacloprid in terms of learning. Imidacloprid was administered in doses ranging from 0.2 to 3.2 mg/l, while doses of 5-OH-imidacloprid ranged from 1.25 to 20mg/l. Both insecticides were fed to bees orally. Sucrose consumption in summer and winter bees was measured to control for insecticide intake by observing the quantity of syrup consumed. The learning performance of summer and winter bees was measured using classical olfactory conditioning of the proboscis extension reflex. The PER was similarly used to analyze the bees' reflex response. Bee mortality was measured by counting the number of bees that died during the study.

Winter bees treated with 5-OH-imidacloprid significantly consumed less syrup, and the highest dose of both imidacloprid and 5-OH-imidacloprid induced mortality in winter bees. Furthermore, high doses of 5-OH-imidacloprid reduced winter bees' reflexes, and the highest dose of both imidacloprid and 5-OH-imidacloprid caused reduced olfactory learning performance. Fewer summer bees died from imidacloprid than winter bees, however, imidacloprid was found to reduce reflexes and learning in summer bees more than winter bees. This study is significant as it considers the effects of the neonicotinoid insecticide imidacloprid, and its lesser studied metabolite, 5-OH-imidacloprid, on both winter and summer bees. The researchers recommend that more studies are conducted to establish a better correlation between insecticide effects in laboratories, compared to effects in the field.

Contribution (100 words):

This article outlines the effects of imidacloprid, as well as its lesser studied metabolite 5-OH-imidacloprid on sucrose consumption, chronic mortality, reflex response, and learning performance for both summer and winter bees. Overall, this study also describes how winter bees are more likely to die of imidacloprid than summer bees, but the surviving winter bees are less influenced by the insecticide. The effects of imidacloprid on summer honey bees are consistent with other literature on the topic. This article was included for its discussion of 5-OH-imidacloprid, and the different effects pesticides have on winter bees compared to summer bees.

Reference:

Guez, D., Zhang, S.W., & Srinivasan, M.V. (2005). Methyl parathion modifies foraging behaviour in honey bees (*Apis mellifera*). *Ecotoxicology, 14*, 431-437. https://doi.org/10.1007/s10646-004-1348-3

Summary (300 words):

This primary article explores the effect of methyl parathion, an organophosphorus insecticide, on honey bee behaviour. While pre-existing research has described the direct risks insecticides pose to honey bees, further research is needed to understand the impact of these chemicals have on honey bees indirectly by way of changes to important behaviours. Accordingly, Guez et al. (2005) examines how foraging behaviour is influenced by exposure to methyl parathion insecticide. Foraging behaviour in this study was tested using an experimental apparatus consisting of a manufactured hive spaced seven meters away from a feeder which provided a sucrose solution. The frequency with which the bees visited the feeder thus provided a foraging frequency. The insecticide was applied topically to the thorax of treated honey bees in doses of either 50ng/bee or 10ng/bee.

Bees treated with methyl parathion significantly visited the sucrose feeder only two thirds as often compared to control bees when initially exposed to a 10ng/bee dose. With time, however, this effect was reversed and visits to the sucrose feeder increased significantly. These results are in contrast to the effects of the 50ng/bee dose, which elicited initially no change in foraging frequency followed by an increase, demonstrating a dose-dependent effect of methyl parathion. This study is significant because it demonstrates that organophosphorus insecticides like methyl parathion have a complex and disrupting effect on foraging efficiency. Additionally, a hypothesis to account for differential foraging rates is presented which suggests that bees forage more because they spend less time in the hive, suggesting less communication amongst foragers. The researchers recommend future studies aimed at uncovering exactly why high doses of methyl parathion result in higher levels of foraging, and if their hypothesis that greater levels of methyl parathion cause a reduction in hive visits is true.

Contribution (100 words):

This article demonstrates that the methyl parathion insecticide influences foraging behaviour in honey bees, and that measuring the rate of foraging offers a convenient and efficient method of analyzing insecticides for their effect on honey bees. Additionally, the results of this study generally align with those of other studies in that insecticides are shown to impact the dynamics of numerous honey bee behaviours, often negatively. This article has been included in this review due to its discussion of methyl parathion, a lesser known insecticide that is distinct from neonicotinoids such as imidacloprid, and because of its discussion of foraging behaviours.